THE UNIVERSITY *of* EDINBURGH

An Investigation into Natural Variation and Adaptation of *Arabidopsis thaliana* in Edinburgh and the Lothians

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Abstract

The use of *Arabidopsis thaliana* populations to understand the genetic basis for natural variation has been highlighted in recent years. The role of adaptation in natural variation remains of key interest. Here, natural variation in growth rate, flowering time and seed production were examined in local populations of *A. thaliana* from the Edinburgh area using a common garden approach. Growth rate and seed production were found to be highly genetically determined and sometimes correlated, and some genotypes were found to perform consistently better as winter annuals and others as summer annuals, suggesting that adaptation to different seasons might maintain natural variation locally. In order to dissect the environmental factors that could affect growth, these genotypes were also grown under controlled conditions. Photoperiod and temperature were identified as two of the seasonal variables to which different genotypes may be adapted. The relationship between growth rate and competition was also examined. In general, competition exaggerated the differences in performance between genotypes, although the identity of neighbours was observed to have an effect on both growth rate and fitness of *A. thaliana* in competition. To understand the genetic basis of growth rate variation, the genetic relationships between local populations was examined. Local accessions were usually found to be more closely related to each other than to world-wide accessions, suggesting that their variation did not reflect recent immigration. To examine the genetic architecture of growth rate variation, hybrids between local genotypes with different growth rates were used in QTL analysis. Four chromosomal regions were detected; these regions represent potential growth-rate associated QTL.

Acknowledgements

First and foremost I would like to thank my supervisor, Andrew Hudson, for offering the wonderful opportunity of such an interesting research project and teaching me of the beauty of natural variation in *A. thaliana*. I have been honoured to be his student, and am grateful for his unconditional support, expert guidance and encouragement throughout my PhD research journey. I also owe a debt of gratitude to my former teacher, Professor Emiratus Joseph P. Mascarenhas, for inspiring my interest in Plant Biology as a naïve young undergraduate.

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While the preliminary work on slug preference did not find its way into this thesis, I owe numerous people a thank you. Firstly, to Gordon Port (University of Newcastle), who provided invaluable tutelage on identifying and finding slug species within the common garden location. Alan Coleman (Linlithgow Academy) and Kirsty Jackson (University of Edinburgh) assisted and collected data as my (temporary) assistants during the trial phase, and I remain immensely grateful for their hard work. I would also like to thank Karen Tieying Lu, who wrote software to automate identification and mapping of slug foraging paths from video data collected during the experiment. Although I was unable to form a full enough set of results for inclusion here, I am duty-bound to acknowledge the tremendous

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My time at Edinburgh was made enjoyable by the many friends I met alongside my study. I very much appreciate Patricia and Sophie's friendship for sharing a lot of emotional burden, as well as their stories and experiences given during my own pregnancy, while writing up this thesis.

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Last but not least, I could not complete this section without acknowledging my little inspiration, baby (now toddler) Juno. The time since her birth (in my 3rd year) has been a mixture of both stress and joy, but I am proud that she will be able to boast to her friends of mama's hard work and achievement.

Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

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Abbreviations

AF	Autumn fast
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AS	Autumn slow
ASF	All season fast
ASS	All season slow
Col	Laboratory accession Columbia
Cvi	Laboratory accession Cape verde
DNA	Deoxyribonucleic acid
dsg	Days since germination
FLC	*Flowering Locus C*
FRI	*FRIGIDA*
FT	FLOWERING LOCUS T
GA	Gibberellic acid
GH	Unheated unlit greenhouse
LE	Low elevation
Ler	Laboratory accession Landsberg *erecta*
LD	Long day
LL	Low light intensity
H^2	Heritability
HE	High elevation
HL	High light intensity
NIL	Near isogenic line
QTL	Quantitative trait loci
RGR	Relative growth rate
RIL	Recombinant inbred line
SD	Short day
SSLP	Simple sequence length polymorphism

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Chapter 1 Introduction

Plants are well documented as displaying phenotypic plasticity; the ability to alter their phenotype as an adaptive response to their environment. Phenotypic plasticity is an important strategy for plants, due to their immobility and consequent inability to change environment. *Arabidopsis thaliana*, for example, has been recorded as showing diverse phenotypic characteristics in different environmental conditions; in characters including germination, growth rate, flowering time, morphology and rosette size (reviewed by Koornneef et *al*. 2004).

While the environment plays a role in influencing development, phenotypic variation can also be genetically defined and therefore inherited from parent plants and passed onto progeny. Thus, heritable phenotypic variation can either be adaptive – some genotypes are fitter in certain environments than others – or may convey no fitness effects (neutral). In the latter case, neutral traits may occur effectively at random, in any environment, as there is no selection pressure to either fix or reject these traits in the population.

Climate change represents one of the fundamental challenges to human society in the modern era. All living organisms within the biosphere are part of interacting system, and loss of biodiversity at any level can have widespread effects. Understanding how plants react and adapt to altered conditions will is therefore of increasing importance. Evidence for adaptation in natural populations of *A. thaliana* for example, could provide a better understanding of how plants might react to environmental change.

Natural history of *Arabidopsis thaliana*

Arabidopsis thaliana is a popular model organism for the studies of adaptation, genetics, evolutionary and developmental biology. First discovered by Johannes Thal (for whom the species is named) in the late Sixteenth Century in the Harz Mountains, northern

Germany, it belongs to the mustard family (Brassicaceae, formerly Cruciferae). The genus *Arabidopsis* comprises nine species and eight subspecies, where *A. thaliana* can be distinguished by various morphological characteristics (i.e. fruit and seed) (Al-Shehbaz and O'Kane 2002). *A. thaliana* is frequently found in diverse habitats (i.e. disturbed areas, sandy soils or river banks) and ranges across most of the world including Eurasia, Africa, and the Americas at various elevations up to 4250 metres above sea level (Al-Shehbaz and O'Kane 2002).

A. thaliana accessions have traditionally been classed as either winter annuals, which germinate in autumn, undergo winter as rosettes and, flower and fruit in spring, or as summer annuals. Summer annuals, also referred to as rapid cyclers, germinate and fruit within the same season (reviewed by Shindo *et al*. 2007; reviewed by Simpson and Dean 2002). Northern European *A. thaliana* accessions, such as those found around Edinburgh and the Lothians, are typically biennial. It is commonly reported that *A. thaliana* is a highly self-fertilized species, with a low outcrossing rate of about 0.3% (Abbot and Gomes 1989). This is associated with small, unattractive and little-scented flowers (low pollinator visitation), which is typical for inbreeding plants (Charlesworth and Vekemans 2005).

Global distribution and collections

A. thaliana is often described as native to Eurasia (Al-Shehbaz and O'Kane 2002), with a genetic gradient from west to east. The greatest diversity has been noted at the western end of the native range (the Iberian Peninsula and North Africa), with the most uniform regions found in the eastern edge of this range, in Central Asia. These observations are consistent with the suggestion that *A. thaliana* populations in the west are the oldest; with later expansion occurring in the eastern end of the native distribution, and within recently colonized regions in the center of the distribution range - such as the Alps (Platt *et al*. 2010; Beck *et al*. 2008, Picó *et al*. 2008; Norborg *et al*. 2005; Sharbel *et al*. 2000).

Recent evidence also suggests that *A. thaliana* from Europe invaded other continents such as North America and Australia since the time of European colonization (Jørgensen and Mauricio 2004; reviewed by Alonso-Blanco and Koornneef 2000). Generally, *A. thaliana* has been found to show isolation-by-distance with geographically closer individuals tending to be more closely related genetically. This suggests that seed and pollen dispersal is limited. However, some local patches of *A. thaliana* individuals in continental Eurasia have been found to consist of identical multilocus genotypes, suggesting that local colonization can occur from a single seed (Bomblies *et al*. 2010; Platt *et al*. 2010; Picó *et al*. 2008). In Britain, one specific genotype was found in many places (a similar observation is noted for North American accessions), which suggests that some genotypes have spread rapidly without inter-breeding (Platt *et al*. 2010).

Details of the biogeography of *A. thaliana* have been described; low temperatures in spring and autumn and high average monthly temperature (>22ºC) with low precipitation in summer were observed to limit its distribution range (Hoffman 2002). Nonetheless, *A. thaliana* can be found from 68ºN (North Scandinavia) to the equator (mountains of Tanzania and Kenya) (reviewed by Koornneef *et al*. 2004). This impressive latitudinal range makes *A. thaliana* suitable for analyzing variation in adaptive traits.

The vast majority of the few hundred characterized *A. thaliana* accessions from western Europe are available in the stock centres (i.e. Arabidopsis Biological Resource Center, ABRC; Nottingham Arabidopsis Stock Center, NASC; RIKEN Bioresource Center, BRC). The collections have been substantially expanded, with more than 2,000 genotypically distinct accessions having been described recently (Bomblies *et al*. 2010; Platt *et al*. 2010; Beck *et al*. 2008; Picó *et al*. 2008).

A. thaliana around Edinburgh

A. thaliana is native to the Edinburgh area, where they grow primarily as biennials. They are more often found to inhabit dry ground, such as on limestone pavement, cliff, screes

and skeletal soils over rock around rural areas as well as built up areas and gardens (PLANTATT, The Royal Botanic Garden Edinburgh).

In this study, thirteen populations of *A. thaliana* were sampled around a 10 kilometre transect from the Kings Buildings (University of Edinburgh) to the Pentland hills, at elevations ranging from 62 to 249 meters above sea level. A number of other plant species such as *Cardamine hirsute*, *Epilobium montanum* and *Poa annua* etc. were often found as neighbours at most sampling sites.

Linkage disequilibrium and polymorphism in *A. thaliana*

A wide range of genetic and phenotypic variations can be found among and within natural *A. thaliana* populations. Although *A. thaliana* populations were traditionally regarded as a consisting of inbred sibships, high genetic variation has been reported by Nordborg *et al.* (2005) and Bakker *et al.* (2006) within local populations collected globally.

Nordborg *et al.* (2002) discovered very little linkage disequilibrium within the global populations studied. Linkage disequilibrium can be defined as the non-random association of alleles or genetic markers in haplotypes. That is, it measures when genes occur together more often than expected by random chance (Abecasis *et al*. 2005; Nordborg and Tavare 2002). Linkage disequilibrium is broken down by recombination between different haplotypes (Nordborg *et al*. 2005). It can therefore inform upon the recombination history of a particular population, particularly for a population which is highly inbred (Abecasis *et al*. 2005; Nordborg and Tavare 2002).

Hence, the selfing nature of *A. thaliana* should cause high linkage disequilibrium; Nordborg's findings contradict this and suggest that the ancestral haplotypes have been broken down by recombination. Bakker *et al*. (2006) also suggests that cross pollination

may occur to allow gene flow across populations. Low linkage disequilibrium also implies that different haplotypes can occur within populations.

Polymorphism is where individuals in a population have more than one allele appearing at a particular locus. It is important to the process of evolution as polymorphisms are created by mutation and their frequencies can be affected by natural selection as well as non-selective sources such as genetic drift. Through identifying polymorphism in functional genes, possible selection pressures and resultant adaptation can be identified (Shimizu and Purugganan 2005). Diverse genetic polymorphisms in functional genes have been recorded in wild and domesticated plants, including *A. thaliana* (Alonso-Blanco *et al*. 2005)

Nordborg *et al*. (2005) reported a general pattern of polymorphism in *A. thaliana* consistent with that expected for a widely distributed, sexually reproducing (i.e. outcrossing) species. In addition, Clark *et al.* (2007) re-sequenced twenty wild accessions of *A. thaliana* and identified hundreds of thousands of single nucleotide polymorphisms with large effects on gene integrity.

The studies in natural variation

The boom of natural variation studies in *A. thaliana* is mostly driven by the availability of natural populations both in the wild and from collections in stock centres. Numerous studies using wild *A. thaliana* in either controlled or natural environments, or both, have provided important information on phenotypic expression and the potential role of environmental factors in adaptation (i.e. Ågren and Schemske 2012; Fournier-Level 2011; Hancock 2011; Wilczek *et al*. 2009). For example, to determine whether natural populations of *A. thaliana* were locally adapted to spatially coarse-grained environmental variation and whether photoperiod regime per se was responsible for local adaptation, Banta *et al*. (2007) examined a number of *A. thaliana* populations collected from three latitudinally different regions of Europe (northern Spain, The Netherlands, and southern

Sweden). These plants were grown in growth chambers with different photoperiodic regimes representative of three points along the latitudinal gradient from which the plant material was sampled. Banta *et al*. (2007) also included a large number of foreign accessions aiming to test the hypothesis that local plants were better adapted to the local photoperiodic regime than foreign plants. Several traits were found to be correlated with latitude (e.g., bolting occurred later in more northern accessions), consistent with local adaptation. However, plants did not have higher fitness in the growth chamber set to their native photoperiodic regime. It was noted that the possibility remains that photoperiod is used as a proxy for other seasonal environmental variables such as precipitation or temperature, as these were not simulated in the experiment.

Natural populations experience environments that vary spatially and over time. In order to properly understand the effect of variation upon overall fitness of a wild plant, examination on the impact of the natural environmental conditions upon the populations is essential. Study of natural variation using only laboratory accessions or under controlled conditions alone may mask the effects of genes which would otherwise be more pronounced under heterogeneous environmental conditions. For example, in the investigation of several flowering quantitative trait loci (QTL), Weinig *et al*. (2002) grew recombinant inbred lines (RILs) of *A. thaliana*, developed from a cross of laboratory accessions - Lansberg *erecta* (Ler) and Columbia - under natural environments. Several QTLs that had previously been found to affect flowering time under controlled conditions were found to have little effects in the plants grown in natural environment. Conversely, a substantial number of QTL with major effects on bolting date in the field were undetectable under controlled environment conditions. This study demonstrates how a genetic characterization under controlled conditions could provide only a partial picture of ecologically relevant genetic variation.

Examples of abiotic and biotic influences

Weather conditions, such as temperature, affect plant growth. Two key stresses associated with low temperate are low hydration due to water supply freezing and stress from ice crystallization within tissues (Smallwood and Bowles 2002). For example, *A. thaliana* from higher latitudes are expected to have adapted to a colder climate than those from more temperate regions.

Another example - irradiance, has been observed to cause phenotypic variation that associated with photosensitivity in *A. thaliana* (reviewed by Maloof 2003; Maloof *et al*. 2001). These include hypocotyl elongation, petiole elongation and leaf inclination. Plants respond to light signals using photoreceptors, including red and far red light absorbing phytochromes, blue light-responsive cryptochrome and photopins. For example, the ratio of Red (R) to Far Red (FR) light is altered by reflection from neighbouring vegetation and is used by the plant to detect and respond to competition. A low R:FR ratio indicates the presence of competing vegetation and can prompt adaptive responses to either tolerate or avoid shade (reviewed by Franklin 2008).

As well as abiotic pressures in natural conditions such as temperature and light, plants in natural conditions also face pressure from herbivore predation and pathogens. Resistance genes (R-genes) for example, protecting against specific pathogens or predators are maintained by selection and are generally regarded as beneficial in the presence of the pathogen or predator (reviewed by de Meaux and Mitchell-Old 2003). However, R-genes may have an overall fitness cost which prevents them from become fixed across a population (Tian *et al*. 2003). This is consistent with the high levels of nucleotide diversity found around resistance genes in *A. thaliana*, possibly due to a long term 'evolutionary arms race' with predator herbivores and pathogens (reviewed by de Meaux and Mitchell-Old 2003).

Analyzing natural variation in *A. thaliana* and its genetic basis

Wide variation for morphological and physiological traits can be observed among *A. thaliana* accessions collected from different locations. This has attracted many research groups to this natural variation with the aim of identifying the underlying genes and polymorphisms. From the perspective of ecology and evolution, the focus has been on inferring the mechanisms that generate and maintain this variation, and also to identify which allelic variants are adaptive under specific environmental conditions. Thus, natural genetic variation has been identified for many traits by genotype-phenotype correlations either in hybrids or natural accessions (reviewed by Koornneef *et al*. 2004).

Mapping the genes

Physiological and morphological traits that exhibit a continuous phenotypic distribution, within or among populations, are termed quantitative traits. They are often controlled by multiple loci or quantitative trait loci (QTL) which contribute to the phenotypic variation in varying degrees (reviewed by MacKay 2001). A common subsequent step following the identification of genetic variation is to focus on determining how many loci account for that variation and where they are located in the genome (mapping). QTL mapping is thus an essential tool for studying the genetic basis of natural variation – it identifies the locations and quantitative impact of parental alleles, based on the correlation of phenotypic and genotypic data in a mapping population.

The initial step in QTL mapping is to construct populations segregating for heritable traits; often by breeding homozygous parents with strongly contrasting traits. The resulting F1 hybrids will be heterozygous at all markers and QTL that differ in the parents. The F1 is then used to create a segregating population (often an F2 hybrid population). This population's progeny will usually display a continuous distribution of the trait of interest because multiple parental loci will have been randomly mixed by recombination to give rise to novel allele combination with different quantitative effects.

Recombinant inbred lines (RILs) are also regularly used as mapping populations. RILs are derived from F2 by repeated selfing. RILs represent individual homozygous mosaics of the original parent genomes. Because every locus is practically homozygous, the lines can be maintained indefinitely by self-pollination. RILs are therefore useful for QTL mapping because they allow multiple replicates of the same genotype to be measured to obtain a better estimate of genetically determined effects.

A. thaliana is suited to establish hybrid mapping populations because of its small genome (five chromosomes with approximately 135 Mbp), selfing nature (inbred parents are highly homozygous and inbred lines are easily established) with relatively high recombination rate. In particular, *A. thaliana*'s short lifecycle makes it feasible to generate RILs populations in a relatively short time period.

Several approaches can be used in QTL detection. Linkage analysis in hybrid populations, for example, uses recent recombination events in pedigree to estimate the distances among genetic loci. This is usually suitable only for identifying broad chromosome regions, but not high resolution mapping because recombination events which separate a QTL from a closely linked marker will be rare in a mapping population of manageable size (<500 individuals). Therefore, fine mapping is often carried out by Introgressing a region from one parent carrying a QTL allele into the genetic background of the other parent to create a near-isogenic line. A large number of progeny segregating only for this introgressed region can then be used in fine mapping. An alternative approach is provided by association or linkage disequilibrium mapping. This identifies candidate genes by analyzing a population of unrelated individuals, to identify non-random association of marker alleles and phenotypes (reviewed by Shindo *et al*. 2007). However, it has the disadvantages of being sensitive to population structure (more closely related individuals will share more genes by chance).

Following fine mapping of a QTL, candidate genes can be identified by their known functions, relevant expression and differences in their structure or expression between

accessions differing for the trait. Ultimately, it is important to show the predicted effects of the QTL by a complementation experiment using plant transformation - transfering the particular allele of interest from one parent to the other and vice versa, or to a null background – as proof of QTL functions.

Aims and objectives

This work aims to investigate the genetic and phenotypic variation in local populations of *Arabidopsis thaliana,* to understand how this variation has evolved, and whether it might be adaptive.

Accessions from local populations along a gradient of elevation had been found to differ significantly in a number of traits under controlled conditions, including vegetative growth rate and flowering time. This raised a number of questions, which are addressed in this thesis.

A major question is whether local variation is maintained by adaptation. For this to be the case, the variation should be expressed under field conditions and affect fitness. Some genotypes should also be fitter than others under conditions that vary locally, either in space or in time and different genotypes should be fittest under different conditions. Local genotypes were therefore grown under field conditions at two sites at different elevations and in two seasons and their growth rates, flowering times and seed production recorded and related to environmental differences between sites and seasons.

A large proportion of the variation in growth rate, flowering time and seed production in the field could be explained by genetic differences between local accessions. Growth rate and flowering time affected seed production (a proxy for individual fitness) at least under some conditions. Little evidence was found to suggest that different genotypes were adapted to factors that varied with elevation. However, some genotypes were found to perform consistently better as winter annuals and some as summer annuals, suggesting

adaptation to factors that differ between seasons. To identify which aspects of the environment might be involved, the performance of local genotypes was compared under controlled conditions differing for temperature, day length or light intensity. Generally, competition enhanced the differences in performance between local genotypes, however, the identity of neighbours could also affect the outcomes. These findings were therefore consistent with local variation being maintained by adaptation to environmental factors that varied with season.

The genetic basis for the phenotypic variation observed locally was examined in two ways. Firstly, the genetic relationships between the local accessions were estimated from multi-locus genotypes (amplified fragment length polymorphism, AFLP). This suggested that plants from the same location tended to be genetically more similar to each other than to plants from other locations and to share similar phenotypes. It also suggested that the variation seen locally is not the result of recent immigration.

Secondly, hybrids between local accessions were used in QTL analysis to examine the genetic architecture of growth rate variation. This suggested that multiple loci contributed to local variation, consistent supporting the idea that genotypes could be adapted to different environmental conditions in different ways.

Chapter 2 Materials and Methods

2-1 Plant source and collection

Wild *Arabidopsis thaliana* plants were collected by A. Hudson and summer student, S. Whithall, from 13 sites within a 5 km radius of King's Buildings campus (Fig. 2-1-1A and 2-1-1B). Between 2 and 21 plants from within a 10 m radius at each locality were transferred to the greenhouse (long day and heated) to complete their lifecycle. All seeds collected from these plants were sown and grown for one further generation in the same greenhouse to yield seed stock for genotyping and growth studies. These stocks were coded for identification. As an example, for "1B5" the number "1"represents the locality where the original plant was collected; the letter "B" represents the pot into which the plant was originally transferred and the number "5" represents the fifth plant in the pot. Each coded stock is also referred to as 'family' in the field and chamber experiments. The detailed record of the original 13 localities is summarized in Table 2-1-1.

Site ID	Location	Co-ords	Elev (asl)	Habitat	Pop-Size	Other species
01	U. of Edinburgh, King's Buildings Campus, S. of Swann Blg	N55°55.29 W003°10.266'	70m	Sandy loam. 10° SE-facing. Shaded to N. by building.	10% cover	*Epilobium montanum, Sonchus oleraceus, Senecio jabobaea, Galium aparing, Poa sp., Cardamine hirsute, Polygonum aviculare, Sagine procumbens.*
02	U. of Edinburgh, King's Buildings Campus, N. of Swann Blg	N55°55.306 W003°10.265'	70m	Predominantly gravel with sandy loam. Level. Shaded to S by 7-storey building W by deciduous trees.	<1% cover	*Cardamine*

Table 2-1-1 Summary of 13 wild *A. thaliana* sampled sites (1 of 3). Keys: Co-ords = coordinates, Elev = elevation, asl = above sea level, Pop-Size = population size.

Site ID	Location	Co-ords	Elev	Habitat	Pop. Size	Other species
03	U. of Edinburgh, King's Buildings Campus, S. of Rutherford Blg	N55°55.284' W003°10.237'	67m	Loam with fine gravel, 20° E-facing slope, partially shaded to S by deciduous trees.	15% cover	*Cardamine, Senecio vulgaris, Urtica dioica* seedlings, *Rumex obtusifolius, Sonchus oleraceus, Plantago media, Cerastium fontanum*
04	U. of Edinburgh, King's Buildings Campus, Forestry plots	N55°55.295' W003°10.180'	62m	Loam, level. Shaded to S and E by deciduous trees.	15% cover	*Poa annua, Triticum repens, Myosotis arvensis, Digitalis purpurea, Trifolium repens.*
05	U. of Edinburgh, King's Buildings Campus, S of JCMB	N55°55.271' W003°10.397'	75m	Sandy loam. Level. Shaded to all sides by deciduous trees and shrubs.	80% cover	*Saginia procumbens* & moss majority. *Senecio vulgaris* seedlings, *Cardamine hirsute, Epilobium montanum, Oxalis corniculata.*
06	U. of Edinburgh, King's Buildings Campus, W. of CSEC	N55°55.278' W003°10.509'	78m	Sand and humus washed off paved area. Level. Shaded to E by 3 storey building.	30% cover.	*Polygonum persicaria, Poa annua, Senecio jabobaea, Cardamine hirsute, Rosa* sp seedling.
07	U. of Edinburgh, King's Buildings Campus, W. of CSEC	N55°55.277' W003°10.749'	79m	Silt run-off. Level. Shaded to S by evergreen shrubs.		*Epilobium montanum, Senecio vulgaris, Sagina procumbens, Poa annua, Chenopodium album.*
08	U. of Edinburgh, King's Buildings Campus, W. of CSES	N55°55.282' W003°10.750'	79m	Silty loam with gravel. 10° east facing slope. Shaded to W by shrubs.	5% cover	*Poa annua, Sagina procumbens*, moss.
09	U. of Edinburgh, King's Buildings Campus, W. of SAC	N55°55.270' W003°10.714'	79m	Predominantly coarse (>10 mm) gravel with some sandy loam. Level. Heavily shaded to S and W by deciduous trees.	5% cover	Moss, *Sagina procumbens, Cardamine.*

Table 2-1-1 Summary of 13 wild *A. thaliana* sampled sites (2 of 3). Co-ords = coordinates, Elev = elevation, asl = above sea level, Pop-Size = population size.

Site ID	Location	Co-ords	Elev	Habitat	Pop. Size	Other species
10	Loanhead	N55°52.785' W003°09.306'	148m	Loam with gravel. Level. Shaded to E by wall.	5% cover	*Epilobium montanum, Capsella bursa-pastoris, Cardamine,* thalloid liverwort. **A. thaliana* in flower 18/07/07
11	Hillend	N55°53.260' W003°12.643'	249m	Sandy loam with pine litter. 30° slope facing S. Shaded to N by *Pinus sylvestris.*	20% cover	*Cardamine, Epilobium montanum, Rumex obtusifolius.* *A. thaliana* in flower 18/07/07
12	Liberton	N55°53.260' W003°09.826'	115m	Sandy loam. Level. Base of S-facing wall.	50% cover	*Galium aparine, Poa annua, Stellaria media, Epilobium montanum, Cardamine, Sinapsis (arvensis?) seedlings, Triticum repens, Senecio vulgaris.*
13	Straiton	N55°53.147' W003°09.609'	152m	Sandy loam with fine gravel. Level. Shaded by deciduous shrubs to N.	50% cover	*Poa annua,* moss.

Table 2-1-1 Summary of 13 wild *A. thaliana* sampled sites (3 of 3). Co-ords = coordinates, Elev = elevation, asl = above sea level, Pop-Size = population size.

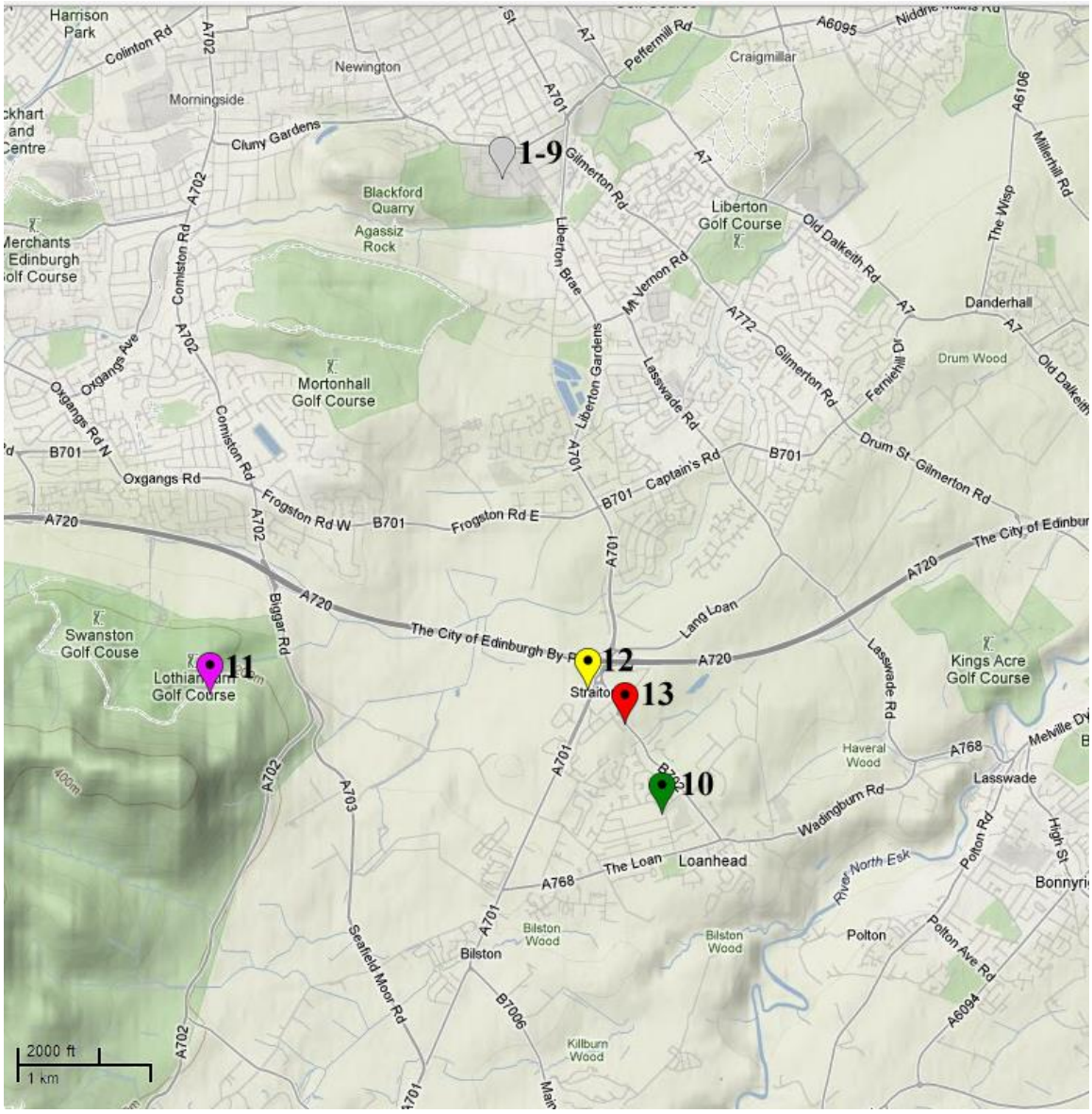

Figure 2-1-1A **Map of sampled sites**. 1 – 9 = localities 01 to 09, Kings' Buildings campus, University of Edinburgh (see Fig 2-1-1B for location details); 10 = locality 10, Loanhead; 11 = locality 11, Hillend; 12 = locality 12, Liberton; and 13 = locality 13, Straiton.

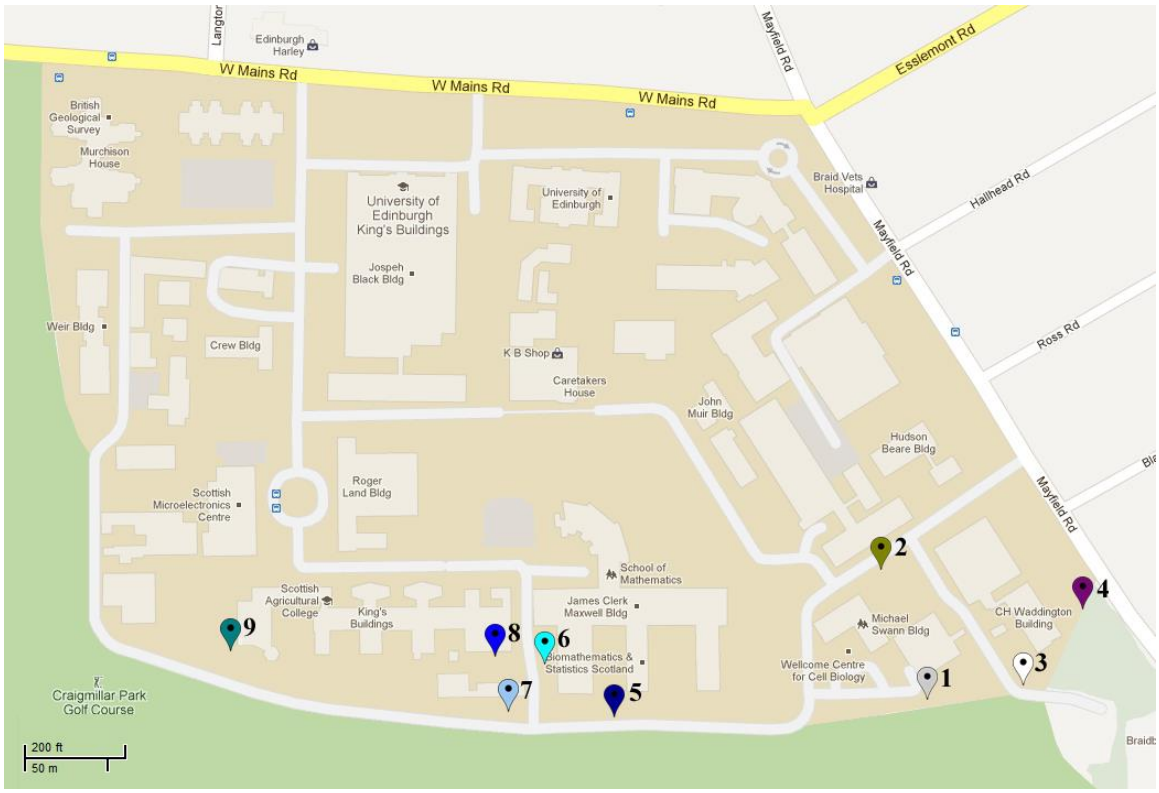

Figure 2-1-1B **Map of sampled sites at King's Buildings**. Keys: 1 – 9 = localities 01 to 09.

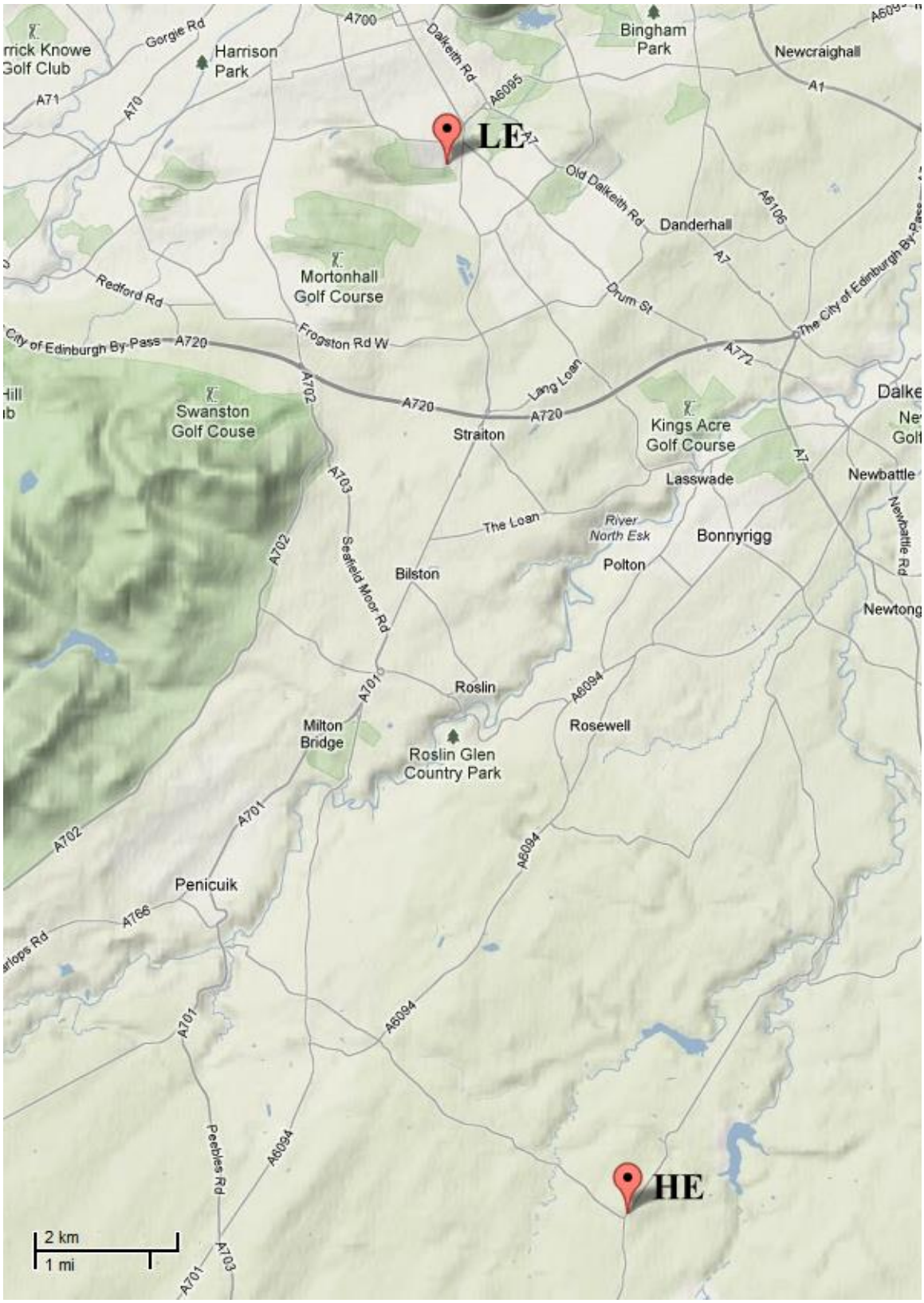

Figure 2-1-1C **Common garden sites**. Keys: LE, low elevation; HE, high elevation.

2-2 DNA extraction

The following genomic DNA extraction procedure was used for all genotyping experiments;

Approximately 0.1 g of leaf tissue was placed in an Eppendorf tube with two 4 mm steel ball bearings and frozen at -80°C overnight. Frozen tissue was ground to fine powder by shaking at 50 Hz in a mixer mill for one minute. DNA extraction buffer (500 µl of 0.1 M Tris pH 8.0, 1.4 M Sodium Chloride (NaCl), 0.02 M Ethylenediaminetetraacetic Acid (EDTA), 2% (*w/v*) Cetyl Trimethyl Ammonium Bromide (CTAB) and 2 µl/ ml of β-mercaptoethanol) was added to each tube of ground tissues. Tubes were shaken at 50 Hz for one minute.

Samples were heated at 65°C for twenty minutes and allowed to cool to room temperature. Chloroform (500 µl) was added to each sample and vortexed. Samples were spun at 17900 x *g* (Eppendorf Centrifuge 5417C) for five minutes to separate aqueous and organic layers.

The clear aqueous layer was transferred into a new 1.5 ml tube. Isopropyl alcohol ($(CH_3)_2CHOH$) (500 µl) was added to precipitate nucleic acids. Nucleic acids were pelleted by spinning at 17900 x *g* for five minutes. The supernatant was discarded and nucleic acid pellet allowed to dry at room temperature. The nucleic acids were dissolved in 40 µl TE buffer (10 mM Tris and 0.1 mM EDTA, pH 7.5) containing 1/1000th volume of 10 mg/ml RNase at 4°C overnight.

To remove ribonucleosides, 1/10th volume of 3 M Sodium Acetate (NaOAc, pH 7.5) and 2.5 volume of absolute ethanol (C_2H_5OH) were added to DNA and mixed well. Samples were spun at 17900 x *g* for five minutes to pellet the DNA. Supernatant in the tube was discarded and pellet was allowed to dry. The DNA pellet was dissolved in 30 µl of TE buffer and its concentration estimated by measuring the absorption of the solution at 260 nm and 280 nm using a Nanodrop spectrophotometer.

2-3 Amplified Fragment Length Polymorphism (AFLP)

Amplification Fragment Length Polymorphism (AFLP) involved two amplification procedures; pre-amplification (section 2-3-4) and selective amplification (section 2-3-5). All polymerase chain reactions (PCR) were run on Peltier Thermal Cycler PTC-200.

2-3-1 Preparation of adapters (50 µM)

Oligonucleotides that were annealed to make adaptors were purchased from VhBio Ltd.

The structure of *Pst*I-adapter:

*Pst*I-adapter forward	5'–CTCGTAGACTGCGTACATGCA–3'
*Pst*I-adapter reverse	3'–CATCTGACGCATGT–5'

The structure of *Mse*I-adapter:

*Mse*I-adapter forward	5'–GACGATGAGTCCTGAG–3'
*Mse*I-adapter reverse	5'–TACTCAGGACTCAT–5'

For annealing adapters, 50 µl of 100 mM solutions of each oligonucleotide (forward and reverse) were mixed in an Eppendorf tube containing 6 µl of 10X OnePhorAll buffer (100 mM Tris-acetate pH 7.5, 100 mM magnesium acetate, 500 mM potassium acetate). The mixture was placed between two heating blocks that have been pre-heated to 95ºC and left to gently cool to room temperature over 90 minutes. Excess annealed adapters were stored at -20ºC.

2-3-2 Restriction digests of DNA

A master cocktail of restriction enzymes for 50 samples contained 100 µl of 5X RL buffer (5X OnePhorAll, 25 mM dithiothreitol or DTT, 0.25 mg/ml Bovine Serum Albumin), 12.5 µl of *Pst*I (10 U/µl, NEB), 6.25 µl of *Mse*I (20 U/µl, NEB), and 256.25 µl dH_2O. For each digestion reaction, 7.5 µl of master cocktail was added to 2.5 µl of genomic DNA (50 ng/µl) and incubated at 37ºC for two hours. The digested genomic DNA mixture was spun at 110 x *g* for twenty seconds and chilled on ice.

2-3-3 Ligation of adapters

A master cocktail of adapters and ligase for 50 samples contained 25 µl of 5X RL buffer, 12.5 µl of annealed *Pst*I adapters, 12.5 µl of annealed *Mse*I adapters, 12.5 µl of 10 mM rATP (NEB), 3.1 µl of T4 DNA ligase (NEB) and 59.4 µl of dH_2O. For each 10 µl digested genomic DNA mixture, 2.5 µl of master cocktail was added. The mixture was spun at 110 x *g* for twenty seconds and incubated at 16ºC overnight.

2-3-4 Preamplification

Universal preamplification primers:

P00	5'–GACTGCGTACATGCAG–3'
M00	5'–GATGAGTCCTGAGTAA–3'

A master cocktail for 50 reactions containing 100 µl of 10X Mg^{2+} free reaction buffer (Biolabs B9015S), 62.75 µl of 25 mM magnesium chloride ($MgCl_2$, Biolabs B9021S), 20 µl of 10 mM dNTPs (Rovalabs), 30 µl of 10 µM P00, 30 µl of 10 µM M00 and 5 µl of Taq polymerase (5 U/µl, NEB) and 502.5 µl dH_2O was prepared at 4 ºC. For each

preamplification sample, 15 µl of master cocktail was added to 5 µl of digested-ligated genomic DNA template. The preamplification reaction was performed using the following PCR cycle profile;

02:00 minutes at 72ºC	---	Initial denaturing (1)
00:20 minutes at 94ºC	---	Denaturing (2)
00:30 minutes at 56ºC	---	Annealing (3)
02:00 minutes at 72ºC	---	Elongation (4)
Cycle steps 2 to 4 for an additional nineteen times		
30:00 minutes at 60ºC	---	Final extension (5)
Cool down to 4ºC	---	End of reaction (6)

For amplification quality control, 4 µl of each preamplified PCR product was mixed with 1 µl of 5X loading dye (NEB) and run on a 2.5% agarose/ 0.5X TBE (4 mM Tris borate and 1 mM EDTA) gel with 0.1 µg/ml of ethidium bromide (EtBr). The electrophoresis was performed at 4 V/cm for 150 minutes and the gel was visualized under UV light to detect products. A representative gel is shown in Figure 2-3-4-1.

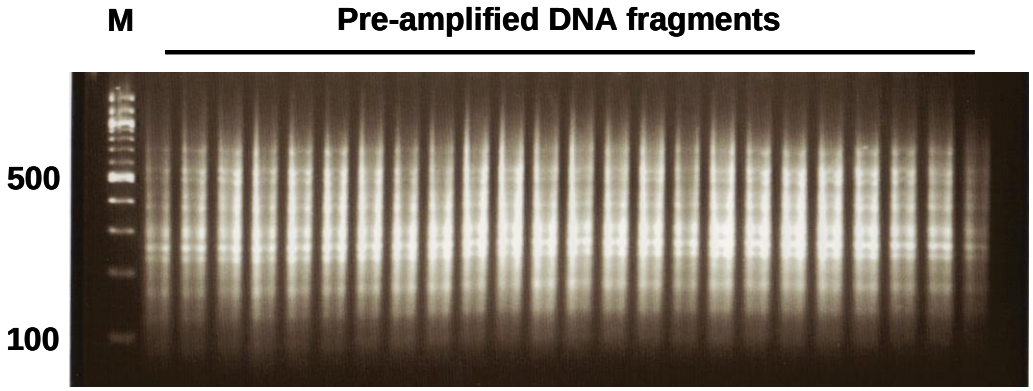

Figure 2-3-4-1 **Pre-amplified PCR products**. Keys: **M** = marker (bp).

2-3-5 Selective amplification

Selective primer pairs;

FAM:			
	*Pst*11 (FAM)	5'–GACTGCGTACATGCAGAA–3'	(*P* primer)
	Mse-CAT	5'–GATGAGTCCTGAGTAACAT–3'	(*M* primer)
VIC:			
	*Pst*12 (VIC)	5'–GACTGCGTACATGCAGAC–3'	
	Mse-ACA	5'–GATGAGTCCTGAGTAAACA–3'	
NED:			
	*Pst*14 (NED)	5'–GACTGCGTACATGCAGAT–3'	
	Mse-ACA	5'– GATGAGTCCTGAGTAAACA–3'	
PET:			
	*Pst*11 (PET)	5'–GACTGCGTACATGCAGAA–3'	
	Mse-ACT	5'–GATGAGTCCTGAGTAAACT–3'	

A master cocktail for 50 samples, containing 50 µl of 10X Mg^{2+} free reaction buffer, 31.25 µl of 25 mM $MgCl_2$, 10 µl of 10 mM dNTPs, 12.5 µl of 10 µM *P* primer (Applied Biosystems), 12.5 µl of 10 µM *M* primer (VhBio) and 2.5 µl of Taq polymerase (5 U/µl) and 331.25 µl ddH_2O, was prepared for each selective primer pair. Preamplification PCR (1 µl) was used as a template with 9 µl of master cocktail for each selective primer pair. All cocktails and samples were prepared at 4ºC and kept out of light. The selective amplification reaction was performed using a touchdown PCR protocol by denaturing DNA at 94ºC (20 seconds), followed by annealing at 66ºC (30 seconds) and finally elongation at 72ºC (2 minutes). The annealing temperature was reduced by 1ºC for the next ten cycles to 56ºC. This touchdown protocol was cycled 20 times.

The following summarizes the touchdown PCR cycle profile;

02:00 minutes at 94ºC	---	Initial denaturing (1)
00:20 minutes at 94ºC	---	Denaturing (2)
00:30 minutes at 66ºC	---	Annealing (3)
02:00 minutes at 72ºC	---	Elongation (4)
00:20 minutes at 94ºC		
00:30 minutes at 65ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 64ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 63ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 62ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 61ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 60ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 59ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 58ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 57ºC		
02:00 minutes at 72ºC		
00:20 minutes at 94ºC		
00:30 minutes at 56ºC		
02:00 minutes at 72ºC	---	Elongation (34)

Cycle steps 2 to 34 an additional nineteen times

30:00 minutes at 60ºC	---	Final extension (35)
Cool down to 4ºC	---	End of reaction (36)

2-3-6 Sample preparation for ABI 3730 Sequencer

The selectively amplified PCR products were diluted with dH_2O; FAM (1:30), VIC (1:100), NED (1:50) and PET (1:50) and 1 µl from each diluted product mixed together. Hi-Di Formamide (Applied Biosystems) with LIZ-500 (Applied Biosystems) size standard was prepared by mixing 1 µl of LIZ-500 with 500 µl of Hi-Di formamide (Applied Biosystems) in the chemical fume hood at room temperature. Finally, 9 µl of Hi-Di/ LIZ-500 was added to 1 µl of the pooled sample mixture. All samples were kept at 4ºC and wrapped in aluminium foil before analysis in an ABI 3037 Sequence Analyser.

2-3-7 AFLP data processing and analysis

Raw data file from the ABI 3730 was converted into .fsa file format by FSA Converter (from University of Wisconsin, Madison Sequencing Service). The converted files were processed by defining reference size standard using GeneScan software (Applied Biosystems). The absence and presence of AFLP bands were viewed and scored using Genographer-2.1 (www.genographer.com). All AFLP bands on digital gel were scored by confirming fluorescent signal intensity.

Nei's genetic distance and Pairwise F_{ST} genetic distance were estimated using AFLP*SURV* (http://www.ulb.ac.be/sciences/lagev/aflp-surv.html; 26-12-2012). A neighbour-joining tree was constructed from the scored data using PAST (http://folk.uio.no/ohammer/past/) and iTOL (Tree of Life v1.0, Ciccarelli FD *et al*. 2006).

2-3-8 Simple sequence length polymorphism (SSLP) PCR

A cocktail mixture of 1 µl 10X PCR yellow buffer, 0.2 µl of 10 mM dNTPs, 0.2 µl of 10 µM forward primer, 0.2 µl of 10 µM reverse primer, 1 µl of 10 ng DNA, 0.4 µl of Tag

polymerase and 7 µl of dH_2O per reaction was prepared. The following describes PCR cycle profile used for SSLP amplification;

02:00 minutes at 94ºC	---	Initial denaturing (1)
00:15 minutes at 94ºC	---	Denaturing (2)
00:20 minutes at 56ºC	---	Annealing (3)
00:45 minutes at 72ºC	---	Elongation (4)
Cycle steps 2 to 4 an additional thirty four times		
05:00 minutes at 72ºC	---	Final extension
Cool down to 4ºC	---	End of reaction

Where necessary, an adjustment was made to the annealing temperature in the PCR cycle profile in order to enhance the reactions.

2-3-9 Agarose gel electrophoresis

To visualize amplification results, 5 µl of each amplified PCR product was loaded on a 3.5% agarose/ 0.5X TBE gel. The electrophoresis was performed at 100 V for 120 minutes and the gel was visualized under UV light to detect products.

2-4 Seasonal Growth Study of *Arabidopsis thaliana*

2-4-1 Germination for seasonal experiments

Seeds were sterilized using a solution containing 70% ethanol (EtOH) with 0.05% sodium lauryl sulphate (SDS) for twenty minutes. The liquid was decanted and 100% ethanol was added to re-suspend the seeds. Seeds were immediately transferred to a sterile filter paper to dry. They were sowed onto ½ MS agar (0.5X Murashige and

Skoog basal salt, 0.6% *w/v* sucrose, 1% agar) plates to synchronize their germination in a light and temperature regulated tissue culture room (24 hours light, 20ºC).

A low concentration of gibberellic acid, GA (25 μM), was used to treat a few families in autumn 2007 set that failed to germinate. These non-germinating seeds were predominantly families from localities 5, 6, 7 and 8. Some families did not germinate after GA treatement, although the remainder germinated successfully.

All seeds were later discovered to germinate well on potting soil (Levington F2 compost), via a simple germination test performed in the field using potting soil (data not shown). As an alternative to GA treatment, seeds were therefore sown directly onto soil in 22ºC LD growth room after sterilization. This method of germination was used to obtain seedlings for experiments starting autumn 2008.

Germination was synchronized for all experiments by sowing seeds at different times. For instance, families from localities 7 and 8 were known to take ten days to germinate, and were sown first. These were followed by seeds from families which took progressively less time, with the sowing date accordingly delayed.

For all field and chamber experiments performed after August 2008, in which the number of families used in the experiment was reduced, sterilized seeds were sown directly onto soil in a 20ºC long day (LD, 16 hour light) growth room.

Three days after germination, seedlings of similar size were selected to grow in the 22ºC LD growth room for one to two days. These were subsequently transferred into an unheated and unlit greenhouse for an additional three to five days.

2-4-2 Selection of experimental sites

A. thaliana was sampled from varied locations across the Edinburgh area, at elevations ranging from 62 m to 249 m above sea level (asl; Fig.2-1-1A & B). Experimental sites were selected at low and high elevations – 67 m and 300 m asl respectively – to reflect the varying elevation of these original sampling sites (Fig. 2-1-1C). Sites were chosen at the extremes of the sampling range in order to better emphasize any differences due to elevation.

Two sites were set up at the low elevation, near the Rutherford Building: low elevation site, LE (67 m asl) and an unheated, unlit greenhouse, GH. A high elevation site, HE (300 m asl) was set up in Midlothian, approximately 7 miles (11 km) from the Rutherford Building (Fig. 2-1-1C).

2-4-3 Autumn/ Winter (Growing from September to June)

Each pot of seedling was carefully thinned to one plant or two plants, in the case of competition experiments, before being transported to the different field sites. A subset of selected plant families (representing every sixth family in the ranking of growth measured under heated greenhouse conditions) was grown in GH.

Pots were placed randomly in trays (eight to twelve pots per tray). All the trays were rotated 180° and shifted weekly, to ensure these plants received a similar amount of light exposure throughout the experimental period. This rotation step was eliminated in later experiments. Plants were photographed from above once a week to monitor growth and development of each individual plant. Once flowering had finished and siliques had ripened, inflorescence were harvested from each individual plant and wrapped in newspaper to dry for later seed collection.

2-4-4 Spring (Growing from March to June)

Seeds were sterilized, sown, planted and grown as in section **2-4-1**. The seedlings were transferred to the same sites as the autumn/winter field experiment.

The positions of pots within a tray were randomized. To aid identification of plants from images, all trays were kept in the same positions throughout the experimental period. Photographic record was taken once a week (twice a week for the set at GH and after mid April for the field sets) to monitor growth and development of each individual plant. The mature inflorescences from each individual plant were harvested as before.

2-4-5 Population size

Table 2-4-5-1 summarizes the population size (number of families) used in all seasonal experiments. The population size in seasonal growth study varied, particularly in the first two seasons; autumn 2007 and spring 2008. The autumn 2007 experiments were performed to gather preliminary data; 39 families were grown in each of the field sites (LE and HE) and a subset of 24 families were kept in GH. Data from this autumn period revealed genetically determined variation in interesting characters such as growth rate, rosette size, leaf colouration and predation rates (from pests). Consequently, a larger set of 72 families was planted in spring 2008. This larger set also had the advantage of addressing variation in seed dormancy discovered in the first autumn.

The number of families in the common garden experiments was reduced to 20 families that represented different behaviours from the beginning of the third season (autumn 2008). Observation of consistently similar phenotypic characters in related individuals sampled from the same locality (relatedness of families are described in Chapter 3) also supported focus upon a reduced set of representative families.

		LE		HE		GH	
		Family	**Ind**	**Family**	**Ind**	**Family**	**Ind**
Autumn	**2007**	39	6	39	6	19	3
Spring	**2008**	69	6	68	6	23	3
Autumn	**2008**	18	12	16	12	20	6
Spring	**2009**	19	9	19	9	19	6
Autumn	**2010**	20	9	20	9	-	-

Table 2-4-5-1 **Summary of seasonal experiments**. LE = low elevation, HE = high elevation, GH = unheated and unlit greenhouse, Family = total number of families in the experiment, Ind = replicate of individuals per family.

2-4-6 Competition

Nine families that had been found to grow better (relative to other genotypes) in autumn, compared to spring (autumn fast, AF), in spring compared to autumn (autumn slow, AS), fast in both seasons (all season fast, ASF) and or slow in both seasons (all seasons slow, ASS) were grown in competitions with each other. Seeds were sterilized and sown as described in section **2-4-l**. Two seedlings were planted 2.5 cm apart from each other in each 7 cm x 7 cm pot. The seedlings were paired with either individuals from the same family or from different families that showed a different response to the season (n = #, 6 to 9 replicates per pairs). A set of seedlings were grown without a competitor as a control group. All seedlings were handled in the same way as described in section **2-4-l**. Photographic record was taken once a week. The relative growth rate and fitness were estimated. These experiments were performed in autumn and spring.

2-5 Chamber study of *Arabidopsis thaliana* growth

Twenty families were used in all chamber experiments. Seeds were sown directly onto soil and the seedlings were handled as described in section **2-4-l**.

2-5-1 Growth in different Photoperiod and Temperature

Two separate sets of experiments were carried out in growth rooms and growth cabinets (five replicates per family). Plants were grown in various temperatures in either long day (LD, 16 hour light) or short day (SD, 8 hour light) conditions (Table 2-5-2-1). Photographic records were taken twice a week for growth rate estimate. Bolting and flowering time were recorded, as well as rosette shape and colour.

2-5-2 Growth in different light intensities

Two sets of plants were grown simultaneously in a highly regulated 20ºC LD growth dome under two light intensity conditions (n = 160 in each condition, eight replicates per family). The average light intensity on the bench top was 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (this is referred to as high light intensity; HL) (Table 2-5-2-1). A 360 cm x 80 cm artificial canopy was made from Benchcote paper and placed 60 cm above the soil surface, in order to create low light intensity (LL) conditions for the seedlings. A single layer of Benchcote was used to reduce light intensity by approximately 85%, without significantly affecting light quality. Trays of plants under LL were rotated twice a week to ensure plants received even exposure. Photographic records were taken twice a week for relative growth rate estimate. Bolting and flowering time were also recorded.

	Growth room				**Growth cabinet**						**GroDome**	
Temp (ºC)	**16**		**20**		**5**		**10**		**16**		**20HL**	**20LL**
Photoperiod	**LD**	**SD**	**LD**	**-**	**LD**	**SD**	**LD**	**SD**	**LD**	**SD**	**LD**	**LD**
Rep per family	3	3	3	-	5	5	5	5	5	5	8	8
No. of family	20	20	20	-	20	20	20	20	20	20	20	20
Total plants (n)	60	60	60	-	100	90	90	90	100	90	160	160

Table 2-5-2-1 **Summary of photoperiod, temperature and light intensity experiments**. The summary of population size, number of replicates (**Rep**) per family and the number of families used in the experiment is shown. Keys: LD = long day (16 hour light), SD = short day (8 hour light), HL = high light intensity, LL = low light intensity.

2-6 Identification of growth rate quantitative trait loci (QTL)

2-6-1 Mapping Population

A fast growing *A. thaliana* parent, 4A6 (ranked 10/56 in previous growth rate ranking under LD conditions) and the slow growing, 9A3 (ranked 44/56) were crossed. The F1 progeny were grown under optimum greenhouse conditions (22ºC, 70% humidity, 16 hour light). The F1 were allowed to self fertilize and 87 F2 plants were grown. F3 seeds were obtained from the F2. They were sterilized and sown on 0.1% MS agar. Then, they were left in the light and temperature regulated tissue culture room to germinate. Seedlings at similar stage of development were selected, transferred to compost and allowed to grow in the LD growth room for two days before being moved to the LD greenhouse. Six individuals of each F3 family were used (n = 507).

Two sets of photographs were taken to determine the relative growth rate of each plant. The first set was taken after the seedlings had grown in the greenhouse for two weeks (having about six leaves on each plant), and the second set was taken a week later. The relative growth rate heritability was estimated and data was analyzed using ANOVA.

DNA was extracted from the leaves of the 61 fastest or 63 slowest growing individuals (in the upper or lower 12^{th} percentile of RGR, respectively). Pooled DNA samples were used for bulk segregant analysis by DNA microarray hybridization.

2-6-2 Microarray Hybridization and Analysis

DNA samples were sheared into fragments between 300 bp and 600 bp by sonication. The sonicated DNA fragments were purified using QIAquick PCR Purification Kit (QIAGEN, cat no. 28104). The two pooled DNA samples were sent to NimbleGen, where they were labelled with Cy3 or Cy5 and co-hybridized onto 50-75mer probes on glass slides. These probes (385K) represent the promoter regions of all *A. thaliana* genes

annotated in the TAIR 6.0 genome assembly, covering a region of 2.5 kb upstream of each gene's transcription start site or the interval to the next gene, if this was nearer than 2.5 kb. The average spacing of probes is 100 bp. The raw microarray hybridization data from Nimblegen were analyzed using ChipMonk (http://www.bioinformatics.bbsrc.ac.uk/projects/chipmonk/). *A. thaliana* gene density was calculated from the TAIR 8.0 assembly using a Perl program written by K. Sujai.

2-7 Rosette area and relative growth rate calculations

The following procedure was used for all experiments conducted;

Rosette areas were calculated from photographic images of trays of plants. Each individual rosette in the image was converted into a black and white binary image using Photoshop. A disc of known area, which had been placed on each tray of plants and photographed, was also converted into a binary image for calibration. Binary images were analyzed using ImageTool software (http://ddsdx.uthscsa.edu/dig/itdesc.html). Rosette areas (in pixels), determined by ImageTool analysis, were converted to cm^2 using the disc as a standard. The relative growth rate was calculated by the change of natural logarithm of rosette area, divided by time (expressed as the number of days) as shown in the following equation:

$$\text{Relative Growth Rate} = \Delta \ln \text{Area} / \text{Time}$$
$$= (\ln A_f - \ln A_i) / n \text{ Days}$$

Where A_f is the final area and A_i is the initial area.

2-8 Statistical analysis

One-way ANOVA and nested-ANOVA were carried out either in Microsoft Excel or PAST. The correlation of rosette areas or relative growth rate between different

elevations (LE versus HE) and seasons from the field experiments were estimated by least-squares linear regression in PAST. Rosette area and growth data were normalized within experiments to allow comparisons between experiments. Some comparisons were made by ranking families within an experiment in an ascending order of growth rate. GxE interactions were estimated using R (http://www.r-project.org; accessed May 2013).

2-9 Heritability

Broad-sense heritability was estimated in each experiment. Broad-sense heritability is abbreviated and referred to as H^2 from here onwards; this nomenclature is as suggested by Allendorf, Luitken and Aitken (2007), in order to distinguish broad-sense (H) and narrow-sense (*h*). The total phenotypic variance, Var_{total}, variance between families, $Var_{between,}$ and variance within family, $Var_{within,}$ were calculated using the following equations:

$$SS_{Total} = \sum [(p1 - \bar{u})^2 + (p2 - \bar{u})^2 + (p3 - \bar{u})^2 + \ldots (px - \bar{u})^2]$$

$$SS_{between} = \sum \{[(\mu 1 - \bar{u})\,(n1)]^2 + [(\mu 2 - \bar{u})\,(n2)]^2 + \ldots [(\mu x - \bar{u})\,(nx)]^2\}$$

$$SS_{within} = SS_{Total} - SS_{Between}$$

Where SS is the sum of squares, *p* is the phenotypic measurement of individual plant, μ is the family mean, $\bar{u}$ is the population mean, and *n* is the number of replicates per family.

$$Var_{between} = SS_{between} / df$$
$$= SS_{between} / (nm\ families - 1)$$

$$Var_{within} = SS_{within} / df$$
$$= SS_{within} / (N - nm\ families)$$

Where *df* is the degree of freedom, *nm* is the number of families and *N* is the population size.

The added variance component among groups, due to random effects arising from differences in genetics and (or) environment, was subtracted from $Var_{between}$ (Sokal and Rohlf 1995) as follows;

$$Var_{\mathbf{Between}} = (Var_{between} - Var_{within}) / \textit{avg per family}$$

$$Var_{Total} = Var_{\mathbf{Between}} + Var_{within}$$

Where *avg* is the average sample size.

$$H^2 \geq Var_{\mathbf{Between}} / Var_{Total}$$

Hence, H^2 approximates to the proportion of phenotypic variance that is due to genetic differences between genotypes.

The value of heritability is depended on the magnitude of all the variance components. A change of any of these components will affect the estimate of heritability (Falconer 1985). For example, heritability can be over estimated because of environmental or maternal effects. Natural environmental conditions specifically, can influence heritability because selection can vary from year to year within a population. Maternal effects are known to contribute substantially to an individual's phenotypic variation (reviewed by Roach and Wulff 1987); the influence of maternal effects can be frequently observed in the birth weight of mammals (i.e. Wilson *et al*. 2004). In plants, strong maternal effect on traits such as seed size and germination has been reported in various plant species, i.e. *Zea mays*, *Brassica campestris* and *Arabidopsis thaliana* (reviewed by Donohue 2009).

Chapter 3 The relationship between local *Arabidopsis thaliana* populations

Introduction

"Isolation by distance", introduced by Sewall Wright (1943), describes the increase in genetic difference between populations with geographic distance. Genetic differentiation arises as gene flow is restricted by the geographic distance between populations and through random genetic drift. Subsequent work has further developed Wright's theoretical analysis, and the theory has been extensively supported by different measures of genetic differentiation (such as estimating genetic distances).

Genetic variation occurs among and within wild populations of *Arabidopsis thaliana*, which inhabits a wide range of environmental conditions across globe. Isolation by distance is almost unavoidable in a species like *A. thaliana*, which has a low migration rate and outcrosses only occasionally, and has established itself across a broad geographic range (Platt *et al*. 2010). A number of genetic diversity surveys on global *A. thaliana* accessions indicate large-scale geographical patterns of diversification (Platt *et al*. 2010; Sharbel *et al*. 2000; Breyne *et al*. 1999; Miyashita et al. 1999; Bergelson *et al*. 1998; Innan *et al*. 1997). Research, however, is comparatively limited in terms of examining variation on a local scale.

A few research groups have investigated genetic variation in wild populations on a regional scale, within the native distribution of *A. thaliana*, such as northern Europe (i.e. Stenøien *et al*. 2005), and regions of presumed recently colonized, such as North America (i.e. Jørgensen and Mauricio 2004). Traditionally, most of these studies consist of one or very few genotypes and found evidence consistent with high selfing and colonization by few seeds. Todokoro *et al*. (1995) and Kuittinen *et al*. (1997) independently showed Japanese and Scandinavian *A. thaliana* populations to be highly inbred. Kuittinen *et al*. (1997), for example, studied genetic variation in quantitative traits from six wild Scandinavian *A. thaliana* populations. They genotyped the sampled populations with 20

allozymes and microsatellites, and found high differentiation between but low variation within populations, which supported *A. thaliana* being predominantly inbred.

Local populations can be highly differentiated, despite geographical proximity. Picó *et al*. (2008) has recently reported that genetic diversity of Iberian *A. thaliana* is geographically structured. They genotyped 268 individuals from 100 wild *A. thaliana* populations (sampled in a region approximately 800 x 700 km^2) and found substantial within and among populations differentiation (as indicated by allele frequencies at microsatellite and single nucleotide polymorphism (SNP) loci, as well as multilocus chlorotype frequencies). This led the authors to suggest that *A. thaliana* in Iberia has a longer demographic history than other global region, and that Iberia appears to be populated by several distinct genetic lineages rather than a single homogenous genetic group of populations.

Bomblies *et al*. (2010) on the other hand, showed evidence for more extensive local variation. They sampled over 1000 individuals from 77 stands (a stand is defined as a single cluster of plants separated from other groups by at least 35 meters) in a region approximately 460 km^2 in Tübingen, southwestern Germany. Samples were genotyped with 436 SNP markers in order to investigate the pattern of relatedness and recombination of local stands. Their results suggested the potential of isolation by distance could be generated at local level among closely spaced genotypes. In addition, outcrossing and recombination within local stands could be extensive, while gene flow was rare between stands. More substantially, they observed considerably higher variation among stands (urban versus rural) with varying outcrossing rate.

Genotyping with amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a versatile and widely used molecular technique for DNA fingerprinting. Developed by Vos *et al*. (1995), this technique combines restriction fragment length polymorphism (RFLP) and polymerase

chain reaction (PCR) and can be summarized in four steps: 1) restriction enzyme digestion of DNA, 2) ligation of adaptors to the restricted sites and pre-amplification, 3) selective PCR amplification, using primers that amplify a sub-set of fragments and 4) acrylamide gel electrophoresis or DNA sequencer analysis to identify products. Two restriction enzymes are generally used – typically one with a 6 bp recognition site and the other with a 4 bp recognition site. Different combinations of enzymes can be chosen to give a number of DNA fragments within a particular range.

Most restriction enzymes are sensitive to DNA methylation, a process where a methyl group from a donor molecule is transferred by DNA methyltransferases to either a cytosine or an adenine. A methylation sensitive enzyme will not cut the DNA, or will cut it at a reduced rate, if 5-methylcytosine or 6-methyladenine is present in their recognition sequence (Weising *et al*. 2005). Plant genomes, for example, frequently have MTG and MAG sequences, where M is 5-methylcytosine (5mC) and can also have MG and methylation of cytosines elsewhere.

The restriction enzyme, *Pst*I, which recognizes the sequence 5'…CTGCA↓G…3' is affected by methylation of either C in the recognition sequence. It will not cut DNA if the site is symmetrically methylated at cytosine residues (Knox and Ellis 2001; Tyrka 2002). This may raise an issue in AFLP analysis if DNA samples differ in methylation – for example, different banding patterns were observed when DNA from different developmental stages were used (Weising *et al*. 2005). It has therefore been suggested that DNA template should be obtained from physiologically uniform tissue of the same developmental stage if methylation sensitive enzymes are used. All DNA samples used in this chapter were extracted from whole seedlings at similar developmental stages, which should minimize variation due to methylation. Ultimately, AFLP can be repeated with more DNA sample replicates per genotype.

The use of methylation sensitive enzymes (such as *Pst*I) in DNA fingerprinting, however, may offer certain advantages; for example, *Pst*I is routinely used in AFLP to detect polymorphism in many crop species such as barley, wheat, maize, tomato and rice. The

combination of *Pst*I and *Mse*I restriction enzymes has been found to be more efficient in detecting polymorphism than *Eco*RI and *Mse*I primers in barley (i.e. Powell *et al*. 1997) and wheat (i.e. Peng *et al*. 2000) – the use of *Pst*I allows better genome coverage and less clustering of marker loci compared to *Eco*RI, which is also sensitive to cytosine methylation within its recognition site, GAATTC. There is also evidence that *Pst*I AFLP markers were more randomly distributed across chromosomes and chromosome regions (in many crop species, at least) whilst *Eco*RI AFLP markers clustered mainly at centromeric regions (Castiglioni *et al*. 1999).

A few disadvantages are associated with AFLP. The most common issue is seen with reproducibility that may occur due to reliability of band amplification. This can be caused by variation in DNA quality or changes in reagents, as the technique is highly sensitive to experimental conditions. Another example of error may arise is band size homoplasy (bands are identical in size but are not identical by descent, which possibly arisen from evolutionary convergence), as loci is distinguished only by size.

However, more importantly, AFLP is sensitive enough to detect multiple loci and low levels of variation, as well as able to discriminate between highly related genotypes (Breyne *et al*. 1999). In addition, the DNA fingerprints can be produced by AFLP without prior sequence knowledge of the species. It is a relatively quick and economical technique to perform. To date, AFLP has successfully revealed phylogenetic relationships and genetic diversity in many species, including *A. thaliana* (Koopman 2005; Stenøien *et al*. 2005, Knox and Ellis 2001).

Arabidopsis thaliana from Edinburgh and the Lothians

Different groups of local *A. thaliana* populations sampled from 13 locations (also referred to as 13 sample groups) around Edinburgh and the Lothians were observed to differ in some genetically determined phenotypes. Growth rate of these local genotypes, for example, was studied under various conditions of controlled light intensity. A

significant proportion of the total variance was found to occur between genotypes, suggesting possible adaptation to localized light condition (unpublished data).

The main aim for this chapter is to study relationships between individuals from various groups sampled around Edinburgh area. The information gathered will allow an evaluation of where local genetic variation stems from; whether or not it is a result of local evolution, or from immigration of 'foreign' genotypes into the local populations.

Differences between sample groups at different localities may serve to indicate local adaptation. To consider this, the distribution of such local genetic variation will be examined; for example, how genetic distances between sample groups relate to their geographic distances.

Finally, it is necessary to consider the relationship between phenotype variation and genetic variation; whether or not individuals with similar genotypes to each other are more likely to share similar phenotypes.

Results

Offspring of all 109 individuals sampled from 13 localities around Edinburgh and the Lothian areas were genotyped. Each DNA sample was pooled from three to six seedlings per family; faster growing seedlings were larger at sampling time required fewer individuals for the required weight of material. The DNA for each family was diluted to 50 ng/µl and digested with *Pst* I and *Mse* I.

A total of 273 fragments were detected by AFLP from four different primer combinations. The mean number of fragments per individual was 143, with most fragments ranging in length between 35–200 bases. Figure 3-1 shows fragment samples of families from four different localities: 8, 9, 10 and 11, on four different colour channels, each with a different combination of selective primers. Of the 273 fragments,

224 (82%) were polymorphic. In addition, members from the same locality were often observed to have similar banding patterns. For example, all members of family 10 shared a unique band at 292 bases on the green channel; similarly, most of family 11 shared a unique band at 333 bases on yellow channel (Fig. 3-1).

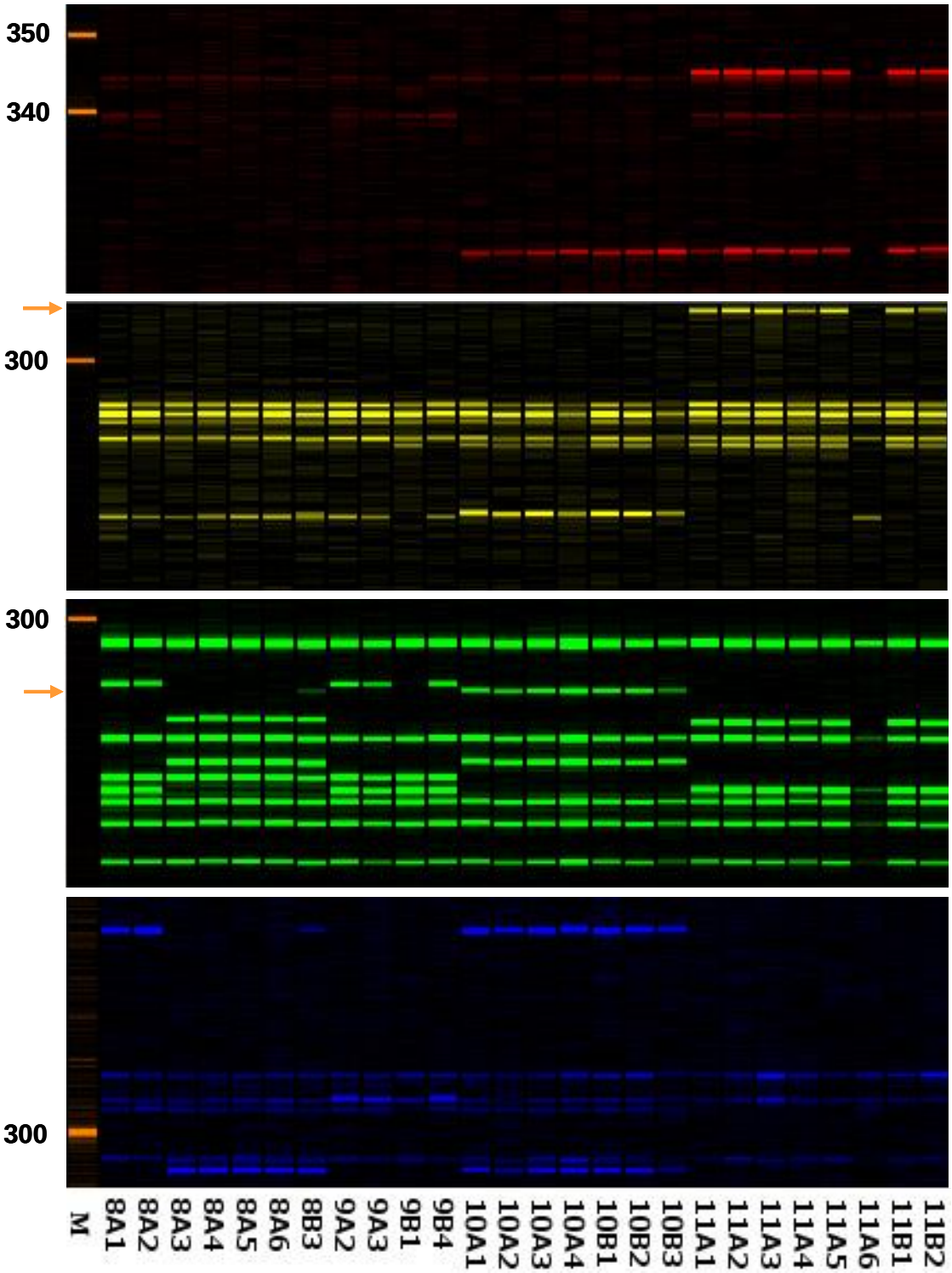

Figure 3-1 **Example of AFLP fragments from four coloured channels**. Polymorphic AFLP fragments were detected throughout the genome of local *A. thaliana*. Keys: **M** = marker (bp); Orange arrows denote 333 bases (yellow channel) and 292 bases (green channel) respectively.

Relationships among local individuals and their gene diversity

The presence of absence of 224 AFLP band was scored in each individual. These were used to calculate the Jaccard distance between individuals, which were then used to estimate a neighbour-joining tree, shown in Figure 3-2. There are several clades consistency solely of plants from one location; for example, those formed only of individuals originating from locality 2, 10, 11 or 13 (Fig. 3-2). The remaining clades contain intermingled individuals from different locations. As an example, the majority of individuals from locality 1 and 4 were found to cluster as a clade, whereas 6, 7 and 8 tended to cluster as another one. Genotypes from locality 5 were widely dispersed and did not cluster together.

Bootstrap analysis (N = 1000) indicated strong support for a number of branches (bootstrap values greater than 95%; Fig. 3-2). For example, clades of families 2, 10, 11 and 13 are supported by bootstrap values over 97%. However, the majority of the branches were supported by weaker bootstrap values (greater than 50%). Low bootstrap values were also detected, often at terminal branching and for outliers (i.e. 5A1, 9B3, 12A1 etc). This implies that the numbers of shared characters supporting each node are small, which in turn indicates that individuals are highly related.

Table 3-1 shows the average expected heterozygosity (Nei's gene diversity) within-population under Hardy-Weinberg genotypic proportions (Hw); Hw was estimated to be 0.1010 ±0.018 (±*SE*) (Lynch & Milligan method, *AFLPSURV*). This provides an estimate of the extent of genetic variability within the sample group; meaning that at any a single locus, there is a 10% probability that any two alleles chosen at random from the population will be different.

The gene diversity among populations was estimated using the Lynch & Milligan method in *AFLPSURV*; providing values for overall gene diversity (Ht), diversity between populations, Hb (or D_{ST}), and Wright's fixation index (F_{ST}; Table 3-1) to serve as unbiased statistical estimates.

Individuals in Figure 3-2 tended to cluster with other individuals from the same site, suggesting that the local populations were differentiated genetically. F_{ST} was used to estimate the degree of differentiation from the proportion of the total genetic diversity that occurs between, as opposed to within, populations (genetic correlation between pairs of genes sampled within a group relative to pairs of genes sampled within the overall set of groups). Sample groups that are not differentiated have F_{ST} values close to 0. The F_{ST} was estimated to be 0.6297 ±0.096 (±SE), which suggests a large genetic differentiation among sample groups (Table 3-1).

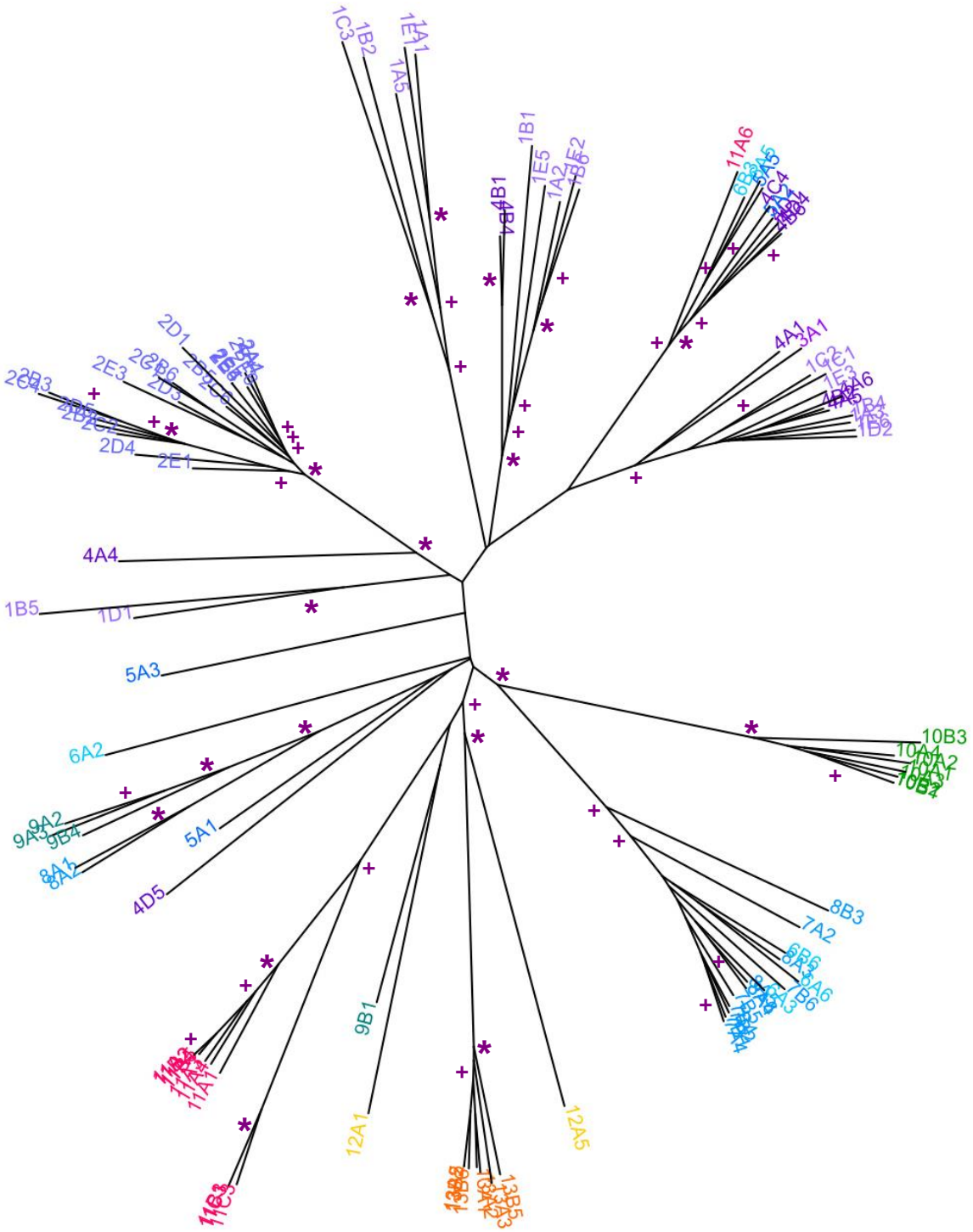

Figure 3-2 **The phylogenetic tree of local *A. thaliana***. This unrooted tree is constructed on the basis of 224 polymorphic AFLP fragments. Families are colour coded for easy visual sample group identification (1 to 13). Refer Fig. 2-1-1 for each group sampling location. Keys: * = bootstrap values greater than 95%; + = bootstrap values greater than 50%.

N pop	Ht	Hb	F_{ST}
13	0.2723	0.1712	0.6297
SE		0.0236	0.0964
Var		0.0005	0.0093

Table 3-1 G**enetic differentiation among sample groups**. Keys: **Ht** = total gene diversity; **Hb** = genetic differentiation among groups (an analogue to Nei's gene diversity within populations, D_{ST}).

Genetic and geographic distance

Genetic distance is an index that measures divergence among populations or species. To test whether genetic differences between local *A. thaliana* individuals were related to their geographic distances, two measures of genetic differentiation were used, Nei's genetic distance (D) and pairwise F_{ST}, and compared to geographic distance.

Nei's D (1972) assumes that differences arise due to mutation and genetic drift. This genetic distance measures the accumulated allele differences per locus. If the rate of gene substitution per year is constant, it will be linearly related to the divergence time between populations under sexual isolation. More importantly, this measure is applicable to any kind of organism without regard to ploidy or mating scheme (Nei 1972).

Wright's F_{ST} measures genetic similarity – it is particularly useful for inferring pattern of gene flow. When it is computed for pairs of populations, it may also provide an estimation of genetic distance among populations, provided that the populations are not interbreeding (which would cause different allele frequencies to those of the total population). Pairwise F_{ST} is useful in identifying outlier populations; i.e. those without any genetic relationship to the rest of the population(s) under comparison. The values of pairwise F_{ST} have been suggested as suitable for the use in determining correlations between genetic differentiation and geographic distance (Slatkin 1993).

The correlation between genetic and geographic distance was highly significant (Fig. 3-3); local *A. thaliana* sample groups are more genetically related as proximity increases. The correlation between Nei's genetic and geographic distance was 0.62 ($p < 0.0001$), whereas pairwise F_{ST} genetic and geographic was weaker, 0.54 ($p < 0.0001$).

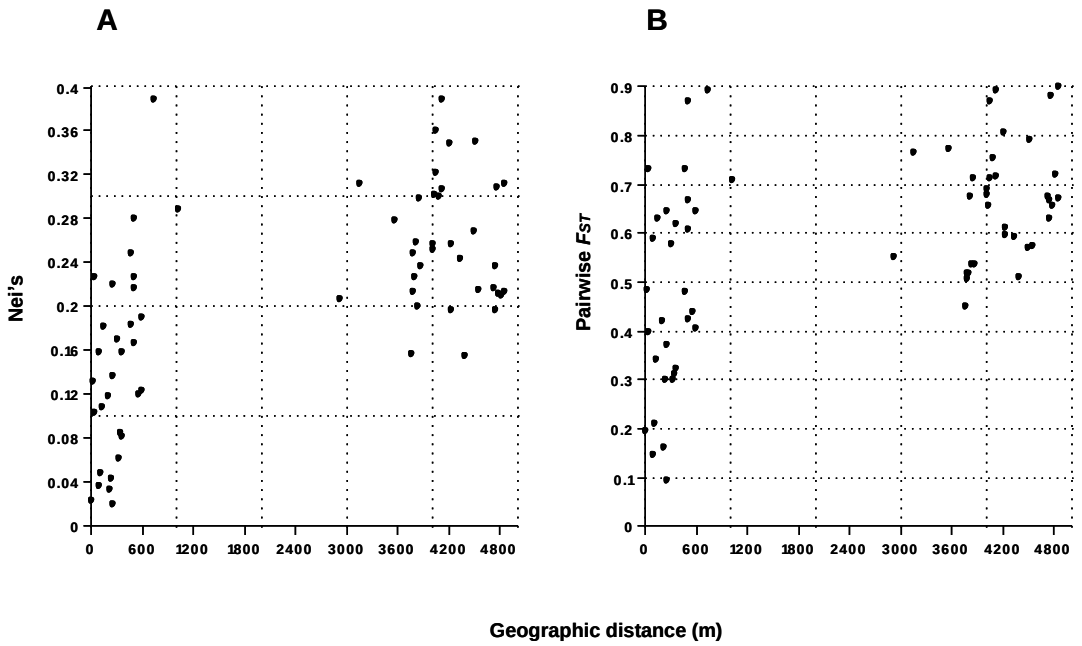

Figure 3-3 **Linear regression for genetic and geographic distance**. Both Nei's genetic distance (**A**) and Pairwise F_{ST} (**B**) correlate with geographic distance, giving r = 0.62 ($p < 0.0001$) and 0.54 ($p < 0.0001$) respectively.

Discussion

The results of this chapter elucidate the relationships within and among local populations through genome-wide DNA fingerprinting.

The local relationships

The genome-wide AFLP based phylogenetic tree provides evidence that the *Arabidopsis thaliana* sampled around Edinburgh are not recent immigrants. If these plants had been recent arrivals, then many plants from the same locality would be expected to have the same genotype or the same genotype to be found at different localities. Instead, most plants were found to be genetically different from each other, but plants from the same location, or from geographically close locations were more similar to each other (Fig. 3-2). This result is unsurprising; *A. thaliana* has been previously reported to have low levels of migration between neighbouring populations. For example, Bergelson *et al*. (1998) detected low levels of genetic variability within but slightly higher among accessions from the estimates of nucleotide polymorphism (one mitochondrial and three nuclear loci) in 11 global accessions. In particular, Bergelson *et al*. (1998) found distinctly different haplotypes in neighbouring populations from within the UK and USA populations, suggesting a relatively low inter-population migration rate.

Many clusters of local Edinburgh genotypes in this chapter were also separated from others by long internal branches (Fig. 3-2). This is in line with the tree pattern which would be expected from an established selfing population. In contrast, a different tree pattern - more rapidly diversified and showing short internal branching with long terminal branches - was reported by Miyashita *et al*. (1999) in a genome-wide AFLP survey of 38 worldwide accessions. The tree topology in Miyashita *et al*.'s study, as suggested by the authors, indicates a recent global spread of the species and since then only limited migration has occurred between populations.

Local sample groups are genetically differentiated, as suggested by Hb and F_{ST} values. As indicated by the phylogenetic tree, genotypes from the same locality origin tend to be most similar to each other (i.e. 2s, 10s, 11s and 13s). They are, however, separated by branches - which in turn indicate that they are not genetically identical. For example, within a cluster a number of branches for individuals from locality 11 are supported by

bootstrap values greater than 50%, indicating a reasonable accuracy of the branches' separations due to genetic variation.

The variation is probably not due to AFLP errors, as local genotypes have been confirmed to be different from each other by microsatellite or SNP genotyping in several cases (unpublished data). The branching pattern is thus likely to reflect mutation or recombination. This would requite these local populations have to have grown around the Edinburgh area long enough to accumulate mutations (the estimated mutation rate for AFLP is approximately 10^{-5}/locus/generation, Korpf *et al*. 2009) or for outcrossing and recombination to occur. Different time lengths of population establishment can result in different genetic diversities within local sample groups; the longer a population is within a particular location, the greater the accumulation of genetic differences from the originals.

Such examples can be seen in the study on local-scaled genetic variation by Bomblies *et al*. (2010). Bomblies *et al*. found low diversity and high homozygosity, as well as no evidence of historical recombination in urban stands; possibly because these plants were usually short lived (rapid local extinction was observed). In contrast, rural stands showed stronger evidence for ancestral recombination due to greater long-term habitat stability. Both urban and rural stands were also found to vary significantly in outcrossing rates - urban stands were mostly selfing, whereas some rural stands could have an outcrossing rate as high as 20%.

The locals and the world

The local groups sampled from Edinburgh and the Lothian are subgroups of UK *A. thaliana* (Fig. 3-4). This was confirmed by SNP genotyping - a subset of local accessions was included along with over 5000 world-wide accessions in genotyping at 149 genome-wide SNP loci (Platt *et al*. 2010). Local Edinburgh accessions were genotyped at up to 139 binary SNPs and 116 were found to be polymorphic. Three haplotypes that are

unique to Edinburgh were detected and found in 9A3, 10B1, 13A1 and 13A5. SNP genotyping at genome-wide loci also showed that three genotypes, 1B5, 1D1 and 4A4, were indistinguishable from a haplotype that occurred UK wide. This could be interpreted as migration, which in turn explains these genotypes being outliers in the phylogenetic tree.

In the majority of cases, the subset of local accessions is genetically most similar to those from Edinburgh. The remainder of local accessions were either most similar to accessions from around Scotland, or from the north of England. This is consistent with differentiation during post-glacial colonization (Platt *et al*. 2010).

Both correlations of genetic (Nei's D or pairwise F_{ST}, Fig, 3-3), and geographic distance, though weakly, indicate a possible isolation by distance. An increase in genetic distance with geographic distance attributed to equilibrium models of isolation by distance has been observed (Slatkin 1993). The correlations between genetic and geographic distance showed that geographic distance is a reasonable predictor of genetic distance of local sample groups. This in turn is consistent with local adaptation to factors that may vary geographically.

In summary, the representation of UK-wide haplotypes suggests migration, whereas the others are more consistent with local evolution. These findings indicate that the natural variation observed in the sampled *A. thaliana* is not due to recent changes. Hence, these populations have been well established at their original locality long to have potentially developed an adaptive phenotype, and are suitable candidates for use in adaptation study.

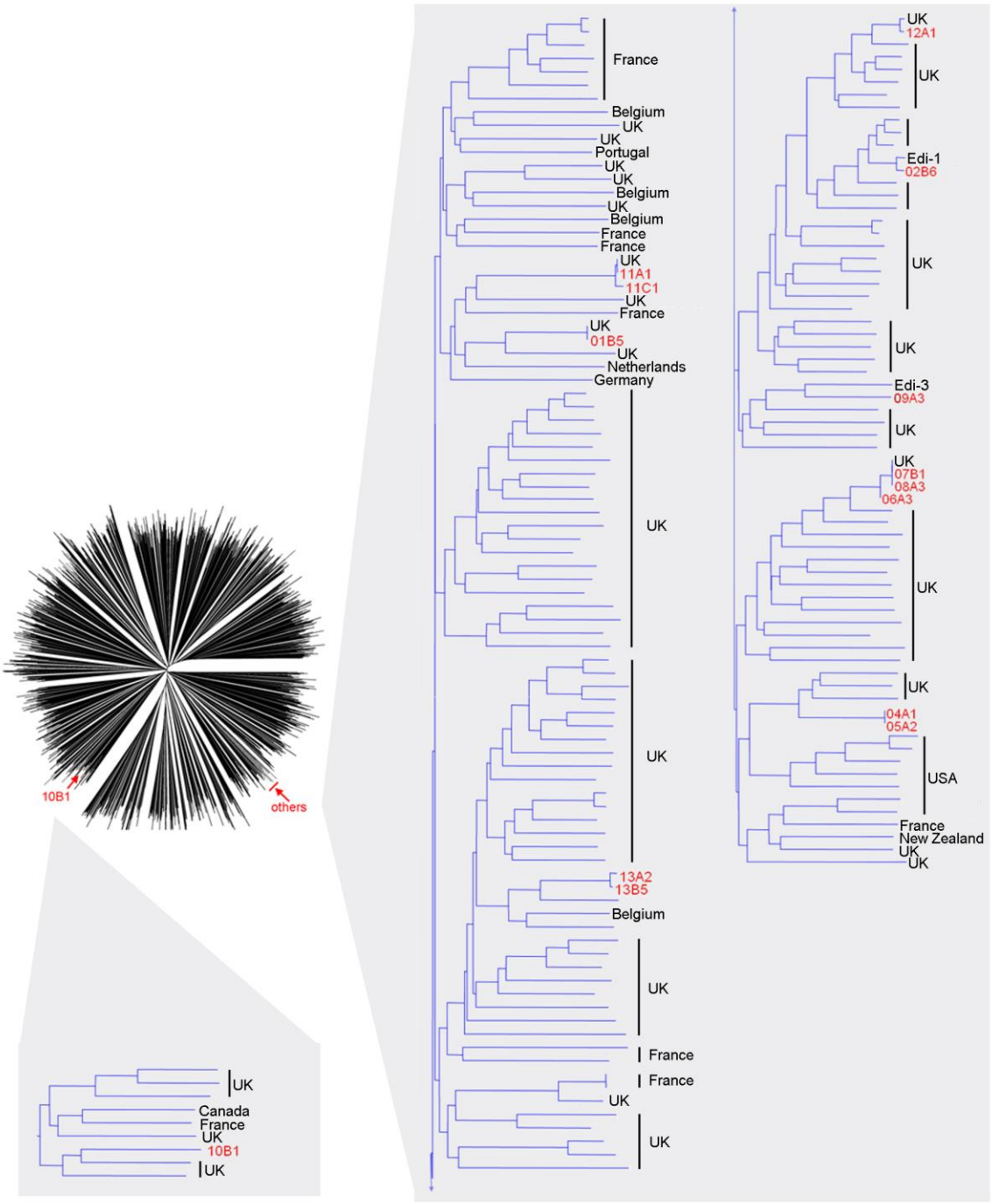

Figure 3-4 **Relationships of local accessions to 5000 world-wide accessions**. Most of the local plants tested, with the exception of 10B1, cluster together with other UK accessions, including previous accessions from Edinburgh (Edi). This is shown as an unrooted tree for all accessions to the left, with clusters enlarged to the right and below, based on genotypes at 149 SNP loci by Platt et al., 2010.

Chapter 4 The weather diary

Introduction

Natural plant populations are often under selection in their natural habitats from both abiotic factors (i.e. climate) and biotic factors (i.e., herbivory and competition). As plants lack the ability to migrate rapidly to relocate themselves in more optimal conditions, their typical response to such pressures is to modify the timing of their germination or growth or reproductive phases (Aitken *et al*. 2008; reviewed by Jump and Peñuelas 2005). Due to ongoing global climate change, as indicated by changing weather trends, there is strong motivation to better understand how abiotic pressures affect existing ecotypes in their natural habitats.

Their relative immobility means that plants offer an ideal subject for studying how climate influences the evolution of organisms. Local adaptation can be observed through recording the fitness changes of plants within a certain area and their correlation to local variation in climate conditions. For example, there is substantial evidence, from studies of fruit yield or at a molecular level (analysis of microsatellite diversity), for the local adaptation within plant species such as *Hordeum spontaneum* (wild barley), *Avena sterilis* (wild oat) and *Triticum dicoccoides* (wild emmer wheat) to local variation in climatic factors such as rainfall and temperature (Volis 2002, 2007; Li *et al*. 2002; Reviewed by Jump and Peñuelas 2005).

A. thaliana offers an ideal candidate organism for studying local adaptation, as it is highly inbreeding – meaning local populations are likely to consist of one or more homozygous genotypes and male and female fitness of individuals do not need to be considered separately.

Two studies of adaptation to climate using *A. thaliana* have also been reported recently. Hancock *et al*. (2011) conducted a genome-wide scans on 948 global accessions and identified a number of single-nucleotide polymorphisms (SNPs) associated with climate

at the collection sites. They also found a strong correlation between fitness and SNP genotypes in common garden experiments in different climates.

Fournier *et al*. (2011) reported a similar study from a common garden experiment (set at four distinctly different locations in Western Europe) using Regional Mapping Panel (RMP) accessions from Finland, Germany, Spain and United Kingdom. They measured plant survival and siliques production in order to estimate individual fitness and its association to climate. A number of SNPs were found to be associated with fitness in a particular environment. Fournier *et al*. also found that fitness at different field sites correlated highly with different loci, suggesting the genetic bases for local adaptation can be environmentally specific.

The aim of the work documented in this chapter is to present a record of local, seasonal weather conditions at the sites in Edinburgh used during the three year experiment period. Edinburgh has a mild annual climate (Met Office 1), despite being situated on a northerly geographical location (55°56′58″N 3°9′37″W). The average annual minimum and maximum temperatures are 5ºC and 12ºC respectively, with a well distributed annual rainfall. Autumn months are normally the wettest, with spring being typically the driest season.

Data from the Edinburgh field sites is used here to identify and discuss the potential roles of key environmental variables upon the growth and development of local *Arabidopsis thaliana* populations.

A focus was placed upon the growth and fitness of individuals, with a secondary focus upon morphological changes potentially attributable to abiotic factors. Analysis of weather patterns and general growth trends was used first to identify the variables most influencing growth rate, and then to attempt to associate them with specific growth patterns.

Results

Weather data were collected from several sources. At low elevation (LE) light intensity and rainfall data were obtained either from the Edinburgh University Geosciences weather station, approximate 220 m due west of the LE and unheated unlit greenhouse (GH) sites or from a small weather station within the field site. Air and ground temperatures at LE and GH sites were recorded with independent data loggers placed between plants. All data from the high elevation (HE) field site were recorded with a small weather station located beside to the plants.

Day length

Day length in the Edinburgh and Lothian areas exhibits a long summer, short winter pattern in hours of daylight (Fig. 4-1), as expected in the Northern Hemisphere. Autumn months (September to December) had an average day length of 10 hours. This shortened to an average of eight hours of daylight through the winter (December to March) and increased to 12 hours in spring and 16 hours in summer. December has the shortest average day length (7 hours) with June having the longest (17 hours). All experiment sites (LE, HE and GH) have the same day length.

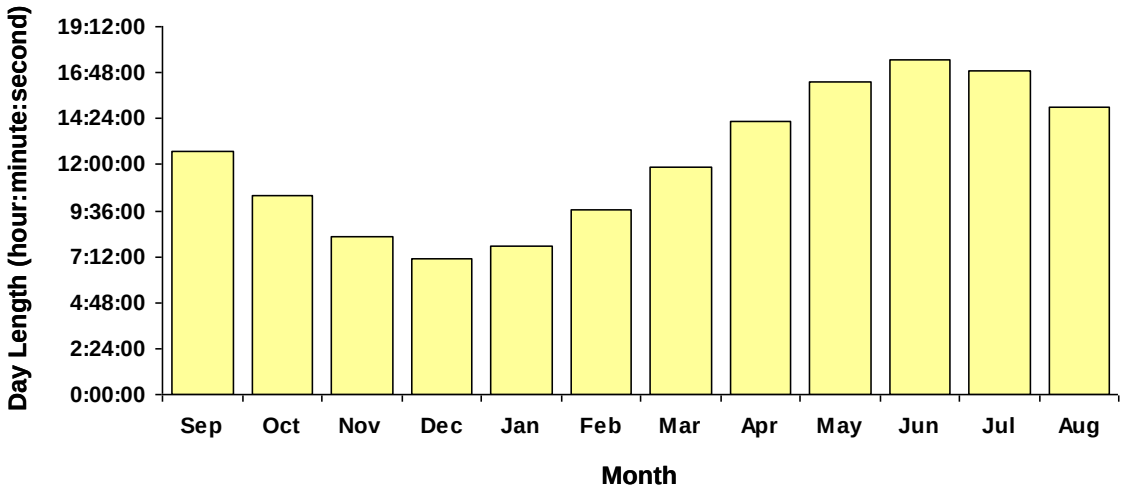

Figure 4-1 **Annual variation in day length**. Monthly average values for day length are shown. Data are shown for September 2008 to August 2009*. The monthly average day length varies by only 30 to 90 seconds from year to year. (*http://www.timeanddate.com/worldclock/astronomy.html?n=304)

Light intensity

Figure 4-2 shows the maximum solar flux (light intensity) from September 2007 to June 2010 at LE. The solar flux has a similar annual pattern to day length (Compare Fig. 4-1 and 4-2). Although solar flux varied slightly between the same month in different years, there was a consistent trend of monthly average solar flux rising from a minimum in December to a maximum in June. The highest recorded solar flux of 0.708 kW/ m^2 was observed during June 2008. This is almost eight times more than December 2008; where the average for the month was only 0.091 kW/ m^2.

In addition, the maximum solar flux measurements at LE and HE sites were similar and highly correlated (r = 0.77 for simultaneous measurements with a regression line of gradient ~1.0), meaning that plants at LE tended to experience similar light intensity to those grown at HE. Variation between the sites can be explained by differences in cloud shading. There was a substantial day-to-day variation in daily total and daily mean solar

flux at both sites but the correlations between sites was higher than for daily maximum (daily total, r = 0.84; daily mean, r = 0.84). Thus, plants at both sites are likely to have experienced very similar levels of light intensity over the periods of the growth study.

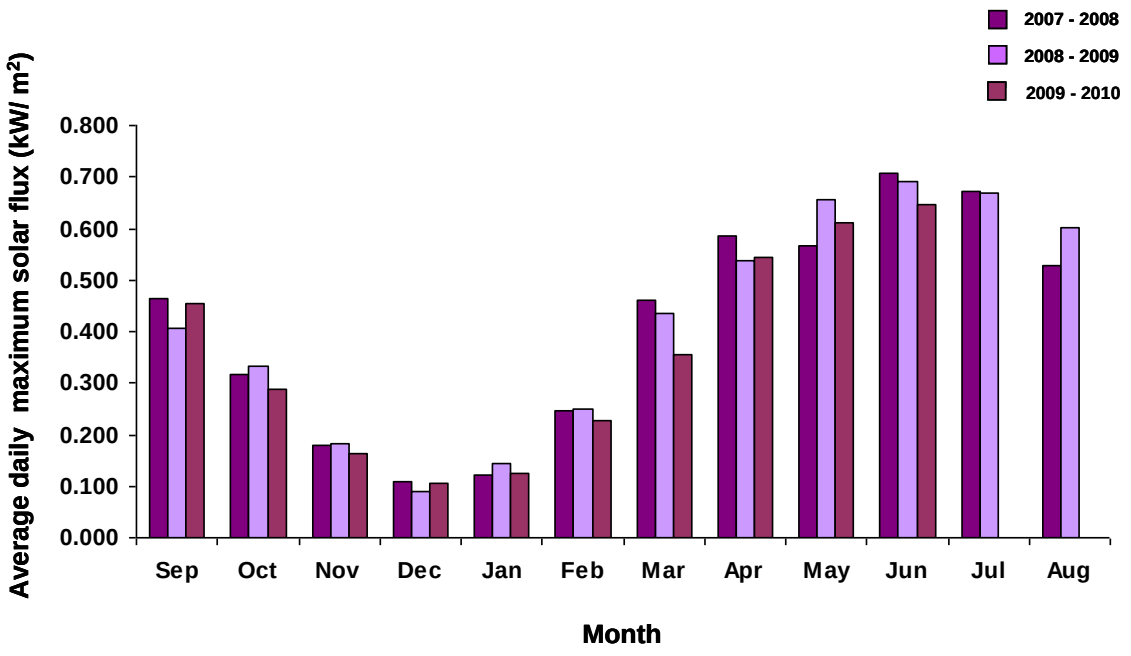

Figure 4-2 **Annual variation in solar flux (light intensity)**. The monthly average for daily maximum solar flux (kW/ m^2) at the LE site are shown. The values for each month represent the years 2007 – 2010 in chronological order, except for July and August 2010, which are not shown.

Air temperature

The annual air temperature fluctuated in a similar manner to day length. At both LE and HE the monthly average for either daily maximum or daily minimum temperature was lowest for December, January or February, depending on the year, rapidly increased during the middle of spring (April and May). Maximum temperature reached its highest value in June but minimum temperature peaked in July or August (Fig. 4-3). The June peak of maximum temperature corresponds to the highest solar radiation and the delay in the peak of minimum temperature can be explained by the time taken for heat to build up in the environment. The average daily temperature was 1.4ºC cooler at HE than LE but

very highly correlated between sites (r = 0.99, where the intercept is 0). The daily maximum (daily max) and minimum (daily min) temperatures at HE were approximately 1.6ºC each lower than LE in all seasons. Again these values were highly correlated as seen in average daily temperature between sites (daily max, r = 0.96; daily min, r = 0.98, where the intercept is 0).

GH temperature showed a similar pattern to LE (Fig. 4-3B). It was approximately 5ºC warmer in the GH unheated greenhouse than outside at LE in autumn and winter, and around 10ºC warmer in spring. The difference average maximum and minimum temperatures at GH were higher than at LE, presumably because sunlight was able to cause rapid heating inside the greenhouse. During winter, the temperature in the greenhouse rarely dropped below freezing point and averaged around 2ºC for the winter months.

The annual range in temperature was similar over the three years of experiments. The LE annual minimum and maximum were approximately 5ºC and 16ºC, respectively (Table 4-1). The minimum and maximum monthly temperatures within a season, however, varied from year to year. In the 2007 and 2008 autumn season monthly temperatures were very similar between years. However, autumn 2009 was warmer than these previous two autumns; particularly during September, which had a 5ºC higher average maximum temperature than the equivalent month in 2007 and 2008.

The weather data also showed a trend of progressively colder winters over the three year period of field experiments (Fig. 4-3A). The spring seasons warmed more rapidly in 2008 and 2009. Spring 2010 was the coldest of the three springs; for March to May the monthly averages for daily minimum and maximum temperatures were approximately 2°C and 7°C cooler than previous spring seasons. However, summer temperatures (starting June) were similar across all three years.

2007 - 2008		2008 - 2009		2009 – 2010	
Min	**Max**	**Min**	**Max**	**Min**	**Max**
5.2	16.8	5.0	16.7	4.0	15.5

Table 4-1 **Annual Temperature Summary**. Annual averages of minimum (Min) and maximum (Max) air temperatures (ºC) at LE.

A

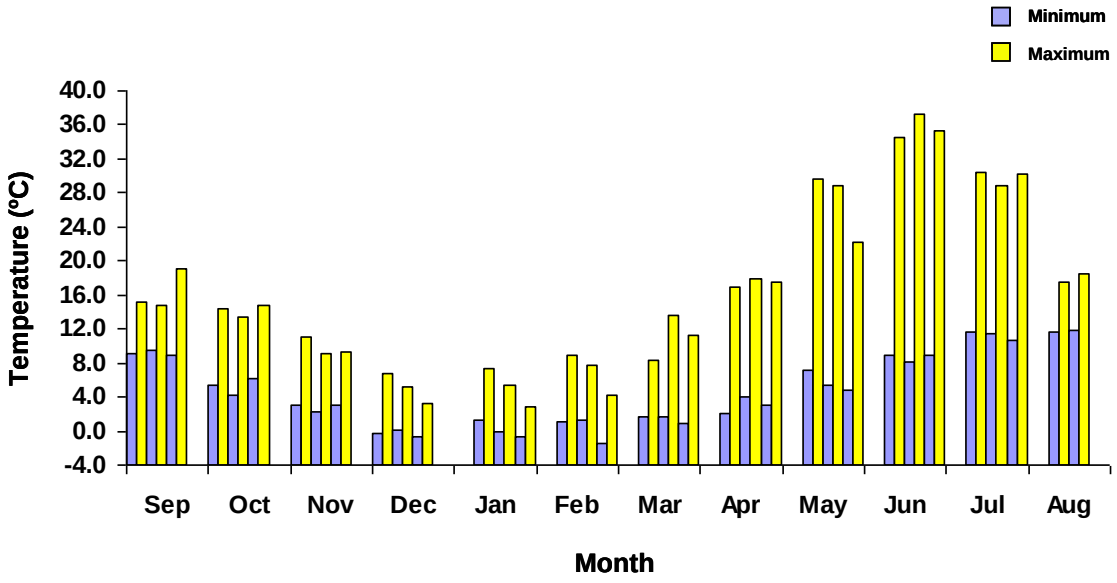

Figure 4-3 **Annual variation in temperature (1 of 2).**

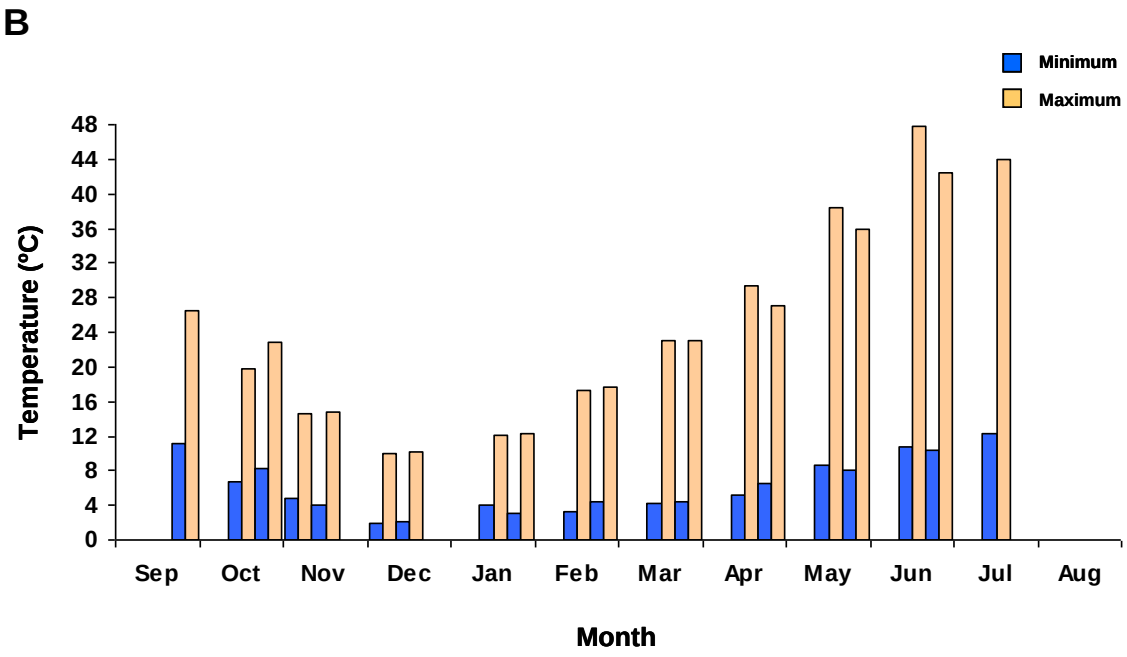

Figure 4-3 **Annual variation in temperature (2 of 2)**. Monthly averages for daily minimum and maximum air temperatures (ºC) at the LE (**A**) and GH (**B**) are shown. Values are for the years 2007 – 2010 at LE (except August 2010), and 2007 – 2009 at GH (starting October 2007 and ending June 2009) in chronological order.

Rainfall

The total rainfall data at LE was obtained from the School of GeoSciences Weather Station, situated approximately 220 m from the LE experimental plot. Although there were large differences in rainfall between the same months in different years, it was observed (Fig. 4-4) that the total rainfall in either autumn (September to November) or winter (December to February) was usually greater than in spring (March to May). For example, 523.4 mm of rain fell in winter 2007, which was over 100% more than spring 2008 (206.0 mm). The relative wetness of autumn and winter differed from year to year; in 2007 winter had significantly more rain than autumn, but the converse was true in 2009. In 2007 - 2008 winter and summer together made up 72% (1039 mm) of the total annual rainfall (1441 mm). With seasonal rainfall less than 200 mm, 2007 had the driest autumn observed during the experimental period (Fig. 4-4).

Rainfall was more evenly spread across autumn (320.6 mm), winter (380.4 mm) and spring (242.8 mm) during the 2008 - 2009 period, compared to the years before and after (Table 4-2). The data also showed significant rainfall in the summers (Su, June to August) of 2008 and 2009.

The autumn-winter period of 2009 had more rainfall (approximately 100 mm more) than previous years. September to November 2009 in particular showed an increasing trend of rainfall with a total seasonal rainfall of 563 mm (Table 4-2). This was followed by the wettest spring of all three years, with 293 mm of rain accumulated: 42% more rain than spring 2008 and 21% more than spring 2009.

Figure 4-5 shows the total weekly accumulation at both LE and HE from March 2008 to September 2009. The data is presented in weekly rather than monthly format due to a number of minor incidents resulting in technical problems with the weather station. Where data was missing for the HE site, these time periods were omitted from the graph due to the lack of comparative data for both sites. Once these disparities were accounted for, there was still a substantial difference of total daily rainfall between LE and HE sites, although these values were correlated ($r = 0.79$). This presumably reflects local differences in weather between sites that are about 15 km apart.

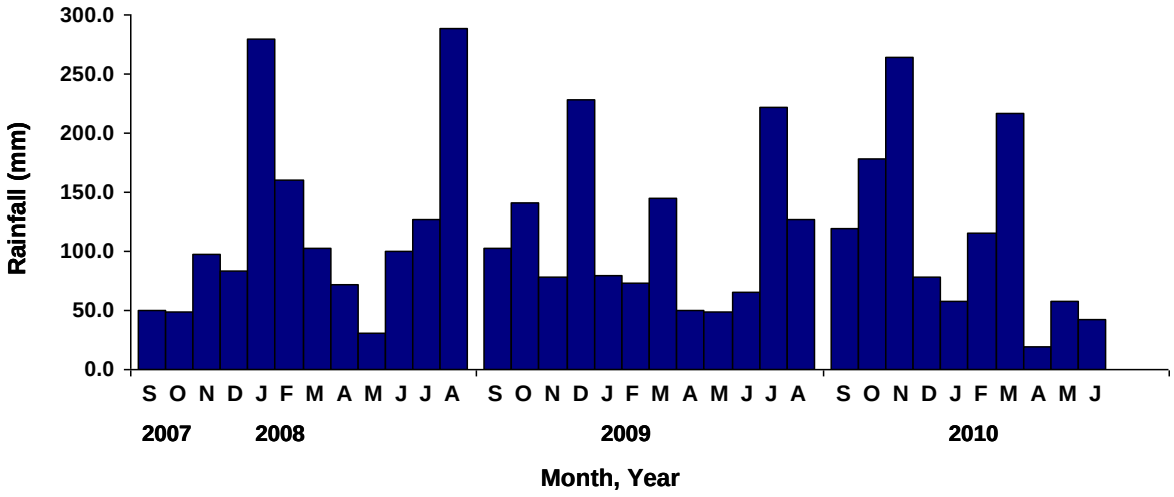

Figure 4-4 **Total monthly rainfall from September 2007 to June 2010 at LE**. Each letter represents a month, beginning with September (**S**) 2007.

2007 - 2008				2008 - 2009				2009 - 2010			
A	**W**	**Sp**	**Su**	**A**	**W**	**Sp**	**Su**	**A**	**W**	**Sp**	**Su**
196.8	523.4	206.0	515.2	320.6	380.4	242.8	414.0	562.8	251.8	293.4	N/A

Table 4-2 **Seasonal rainfall at LE**. The total rainfall (mm) is shown for the four seasons; autumn (**A**; September to November), winter (**W**; December to February), spring (**Sp**; March to May) and summer (**Su**; June to August).

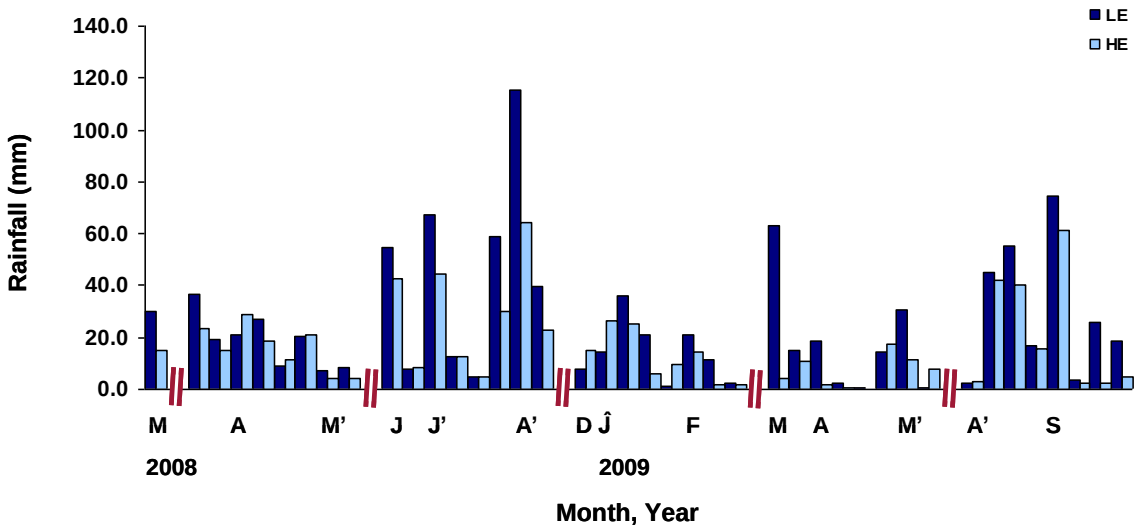

Figure 4-5 **Rainfall at LE and HE**. The total rainfall (mm) at LE (dark blue) and HE (light blue) is shown from March 2008 (local weather station first installed at HE) to September 2009; each letter represents a month, beginning with March (**M**) 2008. Total weekly rainfall (each pair of dark and light blue bars) is presented instead of total monthly as a result of data loss (indicated by double red slashes) caused by individual technical problems with the HE weather station.
Keys: **M'** = May; **J'** = July; **A'** = August; **Ĵ** = January.

Humidity

Humidity is a measure of the amount of moisture in the air; determined by the water vapour resulting from evaporation through irradiance, and transpiration from plants. This is highly influenced by energy (irradiance from the sun) water availability, soil characteristic and vegetation in the area. To a lesser degree, humidity is also affected by wind speed. As a result, humidity tends to be higher in summer months where irradiance and vegetation are substantial.

The daily minimum (min) and maximum (max) air humidity values at LE between 2007 and 2010 was obtained from the Geosciences Weather Station, then used to calculate the monthly average min and max values (Fig. 4-6). The annual averages of daily minimum

and maximum relative humidity were approximately 60% and 88%, respectively. This range difference was consistent across three years (Table 4-3).

The monthly average of daily max humidity remained relatively consistent throughout the year (Fig. 4-6), while minimum humidity was noticeably lower in springs and summers than in winters. The difference between min and max was approximately 20% to 25% in autumn and winter months, but between 30% and 35% in the spring and summer. Nonetheless, each month's humidity range remained relatively consistent across the three years.

There was a substantial variation in daily average humidity levels between LE and HE sites. HE had a higher average humidity than LE (data not shown). Despite the difference, the daily average humidity between both sites was highly correlated (r = 0.74).

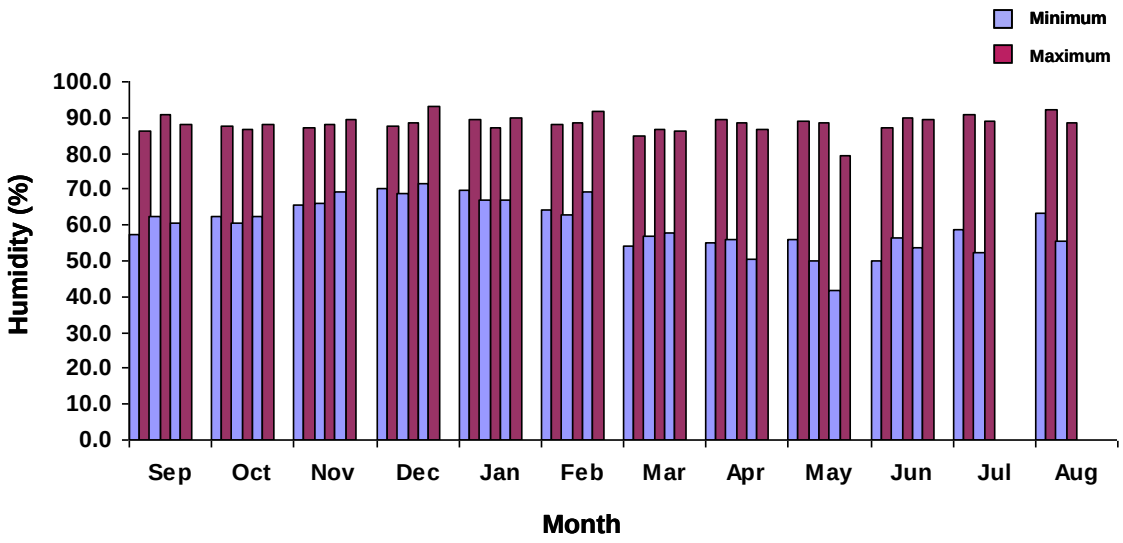

Figure 4-6 **Annual variation in relative humidity**. Average monthly minimum and maximum air humidity (%) values for 2007 to 2010 are shown sequentially for each month, except for July and August 2010.

2007				2008				2009			
Min	***SD***	**Max**	***SD***	**Min**	***SD***	**Max**	***SD***	**Min**	***SD***	**Max**	***SD***
60.5	*±6.37*	88.3	*±1.96*	59.6	*±5.93*	88.4	*±1.18*	59.9	*±9.30*	88.2	*±3.59*

Table 4-3 **Average annual relative humidity values**. Minimum (Min) and maximum (Max) air humidity (%) across three years when field experiments were performed, along with standard deviations (*SD*) are shown. Each annual data set was collected from autumn (September) to summer (August), except for the 2009 data, which only contained humidity records up to June 2010.

Discussion

The collected weather data in this chapter illustrates seasonal weather variation. This is reflected in the observed changes in several key abiotic environmental variables, recorded over different seasons at the different experimental sites. These abiotic variables could be important factors impacting the natural growth in local *A. thaliana*. As expected, most environmental variables varied seasonally, although they differed in the extent to which they varied between the same season in different years – for example light intensity was highly reproducible from year to year whereas rainfall was not. The recorded variables were seen to correlate well between both field sites; although the absolute values naturally varied by location so HE was on average 1.4°C colder and more humid than LE. It is notable that the differences between the two sites for all the environmental variables are less than their seasonal variation at the same site.

The variation of environmental variables: day length, light intensity, air temperature, rainfall and humidity

Amongst the abiotic environmental variables presented in the chapter, day length showed the least variation. There is a predictable annual pattern of short day (photoperiod) during the autumn-winter period and a conversely longer day during spring; this was consistent across all three years in which records were made.

Light intensity has a predictable relationship with day length, and can be seen to follow a similar cycle. Although slightly greater variation between years was seen in light intensity than for day length, autumn-winter grown plans can be reliably expected to receive relatively low light compared to spring-summer equivalents. The observed variations in light intensity between years may be attributable to factors such as the level of atmospheric dust particles and water vapor (including cloud precipitation).

Air temperature showed a similar annual trend. Min and max temperature varied substantially within seasons across the three year experiment period, but within the consistent trend of summer always being warmer than winter. Autumn exhibited more variation; for example autumn 2007 was relatively mild. In 2009, warm summer temperatures were notably prolonged into autumn. Of additional note is that 2007 also had a relatively mild winter, whilst winter 2009 had the coldest average temperature recorded during the experiment period.

The autumns of 2007 and 2009 and the winter of 2009 had a number of unusual warm spells. Some average weekly min and max temperatures were over 5ºC higher than their monthly averages in other years, which ranged from 10ºC to 0ºC at LE. Such warm events, often referred to as 'Indian Summers', were possibly created by the trapped warm air coming from the surrounding warm Atlantic Ocean and seas (Met Office 2).

The occurrence of such warm temperature periods within the otherwise cooler season could perhaps provide 'windows' for continuation of growth. It might also allow opportunities for bolting and flowering during winter, regardless of the actual calendar date

Rainfall was seen to have the least predictable annual pattern. For example, a single month could contribute as much as 20% of the year's total rainfall (e.g., 289 mm for August 2008 from an annual total of 1441 mm), or as little as 9% (127 mm in August 2009 over an annual total of 1358 mm). Nonetheless, local *Arabidopsis thaliana* in

Edinburgh exist within a relatively wet climate, as is common in Scotland. Rainfall levels may vary seasonally, but prolonged drought is unlikely and infrequent. This may mean that the relative importance of rainfall varies depending upon the resource requirements of the plant itself during each growth stage, rather than being shaped by regular periods of scarcity and/or abundance.

Little variation was seen in recorded humidity over the three years of growth experiments. An increase in humidity during winter was observed; this can perhaps be attributed to greater rainfall in the still relatively temperate Edinburgh winter temperatures. Although spring and summer tend to have higher temperatures, these are also typically the driest periods during the year. It is suggested that, in spite of the greater temperatures, the lower rainfall leaves insufficient moisture in the air to increase humidity over that observed in the cooler, but wetter, seasons. This may also explain a higher humidity observed at HE than LE – although HE tended to be cooler, the increased elevation can be associated with similarly increased rainfall. However, it should be noted that rainfall data is insufficiently complete to draw a definitive conclusion to this suggestion. Due to the time period of this experiment, it is thought unlikely that humidity would have had a strong impact upon the growth and morphology of plants grown in the field. Although humidity is known to vary with climate change, generally increasing with temperature (if rainfall remains constant), such variation is unlikely to have occurred during this short experimental period.

In summary, weather patterns remained quite consistent and predictable on a seasonal basis. For example, the average annual maximum and minimum temperature were typically very similar. However, there were relatively unpredictable fluctuations in factors such as precipitation or temperature within the seasons themselves.

Chapter 5 Seasonal growth, flowering time and seed yield of local *Arabidopsis thaliana*

Introduction

Evolutionary adaptation develops as a consequence of natural selection. Adaptation may not always be perfect in nature. For example, natural selection is generally a slow process (depending on generation time, population size, mutation rates and other factors); time lags can cause species to be imperfectly adapted, particularly when selection pressures are changing. Adaptation may also be hindered by genetic constraints. For example, this can occur when the heterozygote at a locus has a higher fitness than either homozygote - homozygotes will always exist in natural populations - hence, the population can never perfectly adapt (reviewed by Hoffmann and Sgrò 2011; Leimu and Fischer 2008; Aitken 2007). Furthermore, adaptation can be hindered by developmental constraints, such as when the same gene affects multiple traits that are selected in different ways. Yet adaptation appears to be a major feature of evolution. Many organisms, for example, appear to be adapted to specific habitat or climate. Penguins are classic example of adaptation within the animal kingdom, from their behaviours (i.e. Emperor penguins hug during winter to keep warm), physiology (i.e. higher levels of myoglobin for storing oxygen during their long dives and heavy layer of fat for cold protection) to physical features (i.e. round short body shape to limit heat loss).

In plants, evolutionary adaptation is not necessarily as immediately obvious as in animals, especially for small weedy species such as *Arabidopsis thaliana*, which often show high phenotypic plasticity. In a heterogeneous environment, the selection pressures upon natural populations are expected to vary across the distribution range (reviewed by Hedrick 2006). Plant populations may display phenotypic plasticity or become locally adapted in response to different environmental pressures.

Local adaptation is defined by improved fitness of a local population within its own habitat (Kawecki and Ebert 2004). Plant species that have a wide geographical

distribution, such as *A. thaliana*, are expected to consist of populations adapted to local climatic conditions (Hancock 2011). This may also lead to a degree of genetic isolation and genetically differentiated plant populations as a result (reviewed by Linhart and Grant 1996).

Nearly a century ago, Turesson (1922) made an important conceptual breakthrough in the study of local adaptation, through demonstrating evidence of genetic differences among local populations of the widely distributed European plant species. For example, Turesson (1925) showed that various Campanula (bellflower) populations, collected from the alpine to lowland of Scandinavian peninsula, Germany, Austria and Hungary, were able to retain their phenotypic characteristics (i.e. flower size and leaf thickness) when transplanted from their original habitat into a common environment. This supported Turesson's hypothesis of genetic variation existed among natural bellflower populations.

Turesson's concept of local adaptation was further expanded by Clausen, Hiesey and Keck. Clausen, Hiesey and Keck (1948) showed that natural *Achillea lanulosa* (wooly yarrow) populations exhibited clinal variation across elevations from 30 m (lowland) to 3000 m (alpine) - populations sampled from different elevations were less fit when grown at other (different elevation) sites. This research resulted in clear indication of adaptation to a specific elevation range and associated climate conditions.

Reciprocal transplant is a highly reliable method of studying and demonstrating local adaptation. It involves comparing the performance of populations when transplanted into the same or a different habitat (Kawecki and Ebert 2004).

Since Clausen *et al*., numerous research teams have demonstrated local adaptation through reciprocal transplants with various plant species, both wild populations (Leinonen *et al*. 2009; Volis 2007; Sambatti 2006; Schemske 1984) and recombinant inbred lines (RILs) (Hall *et al*. 2010). For example, Knight and Miller (2004) demonstrated a small-scale local adaptation using clonal perennial, *Hydrocotyle bonariensis*, sampled within a sand dune (from high and low elevations) on St. George

Island, Florida, USA. They performed a reciprocal transplant experiment by growing *H. bonariensis* at both high and low elevations, where a number of different traits including growth, flowering time (in terms of frequency of flowering) and seed number, were found to be consistent with patterns of local adaptation.

In *Arabidopsis thaliana*, Callahan and Pigliucci (2002) conducted a two-year reciprocal transplant study, along with a parallel greenhouse study, to examine shade avoidance response of natural *A. thaliana* populations sampled from shaded and non-shaded habitats throughout Tennessee, USA. They found differentiation between and within populations for traits such as flowering time and fitness (fruit number); particularly within the greenhouse study, but only weak evidence of local adaptation in the field study. They suggested there may be a trade-off between age and developmental stage that limits the response to selection for adaptive traits.

In another study, Arany *et al*. (2009) investigated the local selection for herbivory pressure on natural *A. thaliana* populations in the Netherlands. The team sampled plants from the coastal region (dune) with high natural herbivory - *Ceutorhynchus atomus* and *C. contractus* (specialist weevils) - and inland (garden), where specialist weevils rarely observed, of the Netherland. Dune site *A. thaliana* populations were known to be natives of the habitat, whereas inland populations were recent colonists. They reciprocal transplanted dune and garden populations and found that dune populations grew better at its home site, with garden populations suffered greater damage from weevils when grown dune site. They did not find significant growth performance differences between dune and garden populations at garden site, suggesting that there maybe insufficient time for garden populations to properly adapt to its environment.

More substantially, Ågren and Schemske (2012) have recently reported evidence of strong adaptive differentiation between natural populations of *Arabidopsis thaliana* from two geographically different regions. In their long term study to assess the magnitude of geographic adaptation, they reciprocal transplanted *A. thaliana* (from seeds) sampled from north-central Sweden and central Italy (which is near the northern and southern

limits of *A. thaliana* native geographic range in Europe) over three consecutive years. The experiments were extended through transplanting seedlings collected from the initial transplant experiment, at the same experimental sites for further two years. Multiple traits, such as survival, flowering time and fitness (seed number per pod) were scored; Ågren and Schemske found that local genotypes were fitter than non-local genotypes in their native environments (i.e. higher survival and greater seed production) across five years. It was also found that the Swedish genotype had higher freezing tolerance than Italian genotype, whilst Italian genotype consistently flowered earlier at both sites. This leads to the suggestion of freezing tolerance may be a major adaptive trait in Sweden. Similarly, early flowering in Italian genotypes may also be an adaptive trait; in this case, to escape summer droughts common in a Mediterranean climate.

An alternative to reciprocal transplants, which uses similar principles, is a common garden approach whereby sites with essential factors, such as soil type, day length, temperature etc., to those of the originals habitats are selected. Many have used this approach for *A. thaliana* (Fournier-Level 2011, Hancock 2011, Wilzcek 2009). When studying climatic adaptation in *A. thaliana*, Rutter and Fenster (2007) performed common garden experiment by planting experimental subjects in a garden environment similar to their habitats of origin. Clinal patterns were tested for, using a distance metric (Gower's environmental distance metric) which scaled with environmental differences between two sites. An inverse correlation was found between fitness, as measured by fruit production, and the distance metric value - i.e. the closer the experimental site was in climate to that of an accession's site of origin, the fitter the genotype would be. This strongly suggests that a common garden approach can be used to identify patterns of climatic adaptation.

Many species exhibit gradual phenotypic and/or genetic differences across a distributional range with varying climate condition. In plants, this was demonstrated in classical experiments that identified clinal variation consistent with local adaptation to differences in elevation (Clausen *et al*. 1948). Additionally, recent work has shown a significant latitudinal cline in one of the major adaptive trait in plants, flowering time,

through common garden experiments with different *A. thaliana* accessions (Caicedo *et al*. 2004; Stinchcome *et al*. 2004). Clinal variation therefore provides evidence for local adaptation; as observed through correlation between these gradual geographical differences (i.e. local climate conditions) and the gradual phenotypic/ genetic variation observed.

Genotype-by-environment interaction (GxE)

Phenotypic traits (i.e. growth, flowering time and seed yield) are influenced by both genes and environment; for example, highly genetically determined phenotypes may additionally be strongly influenced by varying environmental conditions – or vice versa. The term genotype-by-environment interaction (GxE) is used to describe the cumulative impact of genotype and environmental influences upon phenotypes. GxE can reduce the population-level correspondence between genotype and phenotype in a heterogeneous environment. As natural selection acts upon phenotypes, and evolution relies upon genetic changes across populations, GxE may reduce or remove the efficiency of natural selection upon the population when varying environmental conditions act to encourage polymorphism (Gillespie and Turelli 1988). Nonetheless, if individual subpopulations experience different (but consistent) environmental conditions to each other, this can act to encourage local adaptation; genotypes that confer the lowest fitness in each condition will be selected against those with higher fitness. This ultimately leads subpopulations to differentiate genetically and phenotypically, especially when gene flow is low (i.e. due to selfing), as well as migration rate.

Growth

Plant growth is an important component of life history (Bonser and Aarssen 1996). Like many other complex traits (i.e. flowering time and germination), variability in growth can significantly contribute to differences in competitive success across environments,

particularly for short lived annuals (Tremmel and Bazzaz 1995; Weiner and Thomas 1992). Bonser and Geber (2005), for example, demonstrated that rapid growth form (i.e. leaf growth rate and rosette size) evolution is potentially important for adaptation to new or changing habitats. They grew wild-type and mutants of two annual species, *Brassica rapa* and *Arabidopsis thaliana*. Wild-type *B. rapa* produces its leaves on the stem (cauline leaves), while the mutant produces leaves in a basal rosette, mimicking the growth form of wild-type *A. thaliana*. The *A. thaliana* mutant produces more cauline leaves, mimicking wild-type *B. rapa*. It was assumed that the more upright forms would be adapted to lower light levels than the rosette forms. Bonser and Geber, indeed, found that the growth form mutants were often as fit as, or fitter than, wild-type in their predicted optimum environment. The results led the authors to suggest that growth form mutations could result in genotypes pre-adapted to a different environment (in terms of light intensity).

Growth is a complex trait that involves many environmental cues and signaling pathways and involves many genes. Growth analysis such as relative growth rate (RGR) is an important analytical tool to quantify the speed of plant growth over time (Hoffmann and Poorter 2002). Vegetative RGR, for example, is defined as the increase in above-ground biomass per unit biomass per day. Growth rate, and specifically RGR, is a quantitative trait that can be used to characterize the performance of plants within a given habitat or set of environmental conditions. It is a comprehensive measurement that allows integration of the (cumulative) effects of morphological and physiological traits into a single value. RGR is an important component of fitness (McGraw & Garbutt 1990), and it is expected that plants will display a greater RGR in favourable environmental conditions (i.e. those to which they are adapted to).

The involvement of vegetative RGR in *A. thaliana* adaptation, however, has not been studied as extensively as flowering time or seed size. Existing studies have exploited the availability of widely-used global accessions, rather than local accessions, and have used fully controlled laboratory environments, rather than field conditions. In fact, most experiments have used recombinant inbred lines (RILs) grown under controlled

laboratory conditions. For example, El-Lithy *et al*. (2004) mapped a number of quantitive trait loci (QTL) for relative growth rate (involving either leaf leaf RGR or the rate of leaf initiation) in RILs from *Landsberg erecta* x *Shakdara*, whereas Kryomann and Mitchell-Olds (2005) used a near isogenic line (NIL) of *Columbia-0* and *Landsberg erecta-0* to successfully fine-map a candidate growth QTL region as well as identify two epistatic growth rate QTL.

Flowering time

Flowering time is assumed to be adaptively important because it can directly determine a plant's reproductive success (reviewed by Koornneff *et al*. 1998). The transition from vegetative growth to flowering time is governed by endogenous factors and environmental cues factors (reviewed by Amasino 2010). In *A. thaliana*, for example, flowering can be accelerated by vernalization (experiencing a period of cold), warmer ambient temperature and increasing photoperiod associated with seasonal change (reviewed by Simpson and Dean 2002).

Munguía-Rosas *et al*. (2011) performed a meta-analysis on 81 studies that involved flowering time and flowering synchrony, which selection may target. They focused on studies which showed relationships between flowering onset and/ or flowering synchrony and fitness, and reported that flowering time have been selected in many plant species. Their analytical results suggested that phenotypic selection tends to favour early flowering plants, particularly in out-crossing species, which may have several advantages; including more time for seed maturation and a longer growing season for plants produced from seeds that germinate immediately. Selection on flowering time was also found to be influenced by latitude. For some plant species, latitude correlated with flowering time - plants at higher latitudes flowering earlier - rather than other potential influences such as seed predation or pollination.

In another study, Hall and Willis (2006) demonstrated local adaptation in costal and montane populations of *Mimulus guttatus* (yellow monkeyflower) in a reciprocal transplant experiment. They found evidence for divergent selection for flowering: early flowering was favoured at the montane site, where there was a drought period in midsummer, whereas later flowering were selectively favoured at the temperate coastal site, which was almost continually moist.

Some local adaptation studies, such as for *Arabidopsis lyrata* (Leinonen *et al*. 2012) and *Mimulus guttatus* (Hall, Lowry and Willis 2010), have combined reciprocal transplant experiments with QTL mapping to examine the genetic basis for local adaptation. Both Leinonen *et al*. (2012) and Lowry and Willis (2010) separately found evidence supporting conditional neutrality (i.e. an allele shows a fitness advantage in one environment, but is neutral in the contrasting environment) for fitness traits such as flowering time. Leinonen *et al*. (2012) grew F2 populations from reciprocal crosses between two genetically diverged Norwegian and North American *A. lyrata* populations to investigate local adaptation and differentiation in flowering time. They transplanted the hybrids to both parental habitats and found evidence for alleles that conferred earlier flowering and higher fitness in their local environment. They also found that heterosis contributed to increased fitness of hybrids and that interaction between maternally inherited cytoplasmic factors and nuclear QTL affected fitness. Their results showed that local adaptation between diverged populations of *A. lyrata* involved mostly QTL with conditional neutrality, and possibly with putative fitness trade-offs.

Verhoeven *et al*. (2008) explored the patterns of differential natural selection on traits such as RGR, flowering time and seed weight in reciprocal transplantation of two wild barley (*Hordeum spontaneum*) populations. They showed that flowering time variation was a main determinant of fitness in one of the two habitats and identified a QTL underlying this variation. However, they found no evidence for local adaptation involving RGR or seed weight and concluded that the QTL for flowering time is responsible for most of the adaptive population divergence. Flowering plants that grow

over a wide geographical distribution such as *A. thaliana*, will therefore, expected to experience various selection pressures acting upon flowering time.

Optimum flowering time is strongly influenced by climatic signals. The gene *FRIGIDA* (*FRI*) is a key contributor to flowering time variation in natural *A. thaliana* accessions (Simpson and Dean 2002). Functional *FRI* alleles delay flowering, whereas nonfunctional (null) *FRI* alleles allow rapid flowering in the absence of vernalization (Simpson and Dean 2002). Functional *FRI* alleles are therefore thought to be favourable for winter annuals as they delay flowering until spring. Conversely, null alleles should be favourable for summer annuals.

Stinchcombe *et al*. (2004) detected a latitudinal cline in bolting time in *A. thaliana* accessions from different latitudinal origins in a common garden experiment. This was detected using accessions with *FRI* alleles that lacked deletions that would disrupt protein function (putatively functional *FRI* alleles). Accessions from southern latitudes tended to show greater sensitivity to vernalization: possibly as an adaptation to milder winters, which would provide less of a vernalization cue, and hotter, drier summers, which would favour earlier flowering in spring.

In order to investigate the mechanism responsible for Stinchcombe *et al*.'s (2004) observation in latitudinal cline in *A. thaliana* flowering time, Caicedo *et al*. (2004) proposed that selection on *FRI* varies with the *FLOWERING LOCUS C* (*FLC*) genotype. *FRI* up-regulates *FLC*, a MADS-box transcriptional activator that inhibits flowering but is down-regulated by vernalization (Amasimo 2010). Caicedo *et al*. (2004) detected significant differences in latitudinal distribution of the two major *FLC* haplogroups, where one group was predominantly found in northern accessions and the other in the south. Genotypes with the *FRI* deletion would show a reduction in *FLC* activity, and hence would not be seen to express phenotypes associated with *FLC* variation. Caicedo *et al*. (2004) used accessions with putative functional *FRI* alleles and found that latitudinal flowering time variation is associated with epistasis between *FRI* and *FLC*. They hypothesized that the modified protein encoded by the two haplogroups' alternative

transcripts (which differed by a radical amino acid substitution) may lead to differential *FLC* activities, in turn causing latitudinal variation in *A. thaliana* flowering time.

Both *FRI* and *FLC* have also been suggested to account for adaptive differentiation globally. The emergence of nonfunctional *fri* alleles has been reported; two null deletion alleles occurring with high frequencies have been suggested as consistent with positive selection (reviewed by Amasimo 2010). Toomajian *et al*. (2006) evaluated the evidence for recent selective sweeps of two major null *FRI* alleles, fri_{Col} and fri_{Ler} (present in laboratory accessions Columbia, *Col*, and Landsberg *erecta*, *Ler*, respectively), that have been shown to underlie early flowering in many European *A. thaliana* accessions. A selective sweep is where the genetic hitchhiking effect of a beneficial mutation generates a unique distribution of allele frequencies and spatial distribution of polymorphic sites around the selected allele (Kim and Nielsen 2004).

The basis of Toomajian *et al*.'s (2006) approach was chromosomes that are identical by descent at a polymorphic site must also share a short region surrounding that site - the length of this identical by descent region is influenced by the age of the shared allele and recombination rate. Toomajian *et al*. examined pairwise haplotype-sharing (PHS) throughout the genome of 96 global accessions. Their statistical analysis showed extremely high haplotype sharing around fri_{Col} and fri_{Ler} (evidence of selective sweep), leading the authors to suggest these null alleles may have been involved in adaptation, consistent with their effects on flowering time, frequency and geographical distribution.

In a common garden experiment, Korves *et al*. (2007) evaluated selection on *FRI* in the field by examining associations between *FRI* and *FLC* genotypes and fitness traits in 136 European *A. thaliana* accessions. They planted accessions consisting of three *FRI* classes: putatively functional *FRI*, fri^{delCol} (containing16 bp deletion as in the Columbia accession) and fri^{delLer} (containing 376 bp deletion as in Landsberg *erecta* accession) in two different *FLC* backgrounds as described by Ceicedo *et al*. (2004). Seasonal experiments were conducted in autumn and spring. Korves *et al*. found that the fitness effects associated with *FRI* was dependent on both the *FLC* genetic background and

seasonal environment. In autumn, accessions with putatively functional *FRI* alleles had higher winter survival in one *FLC* background; in spring, accessions with null *fri* alleles had greater seed yield in another *FLC* background. Korves *et al*. elegantly demonstrated the impact of *FRI* (putatively functional *FRI* versus null *fri*) in field fitness, as well as highlighting the potential importance of genetic background effects (*FLC*) and the complex patterns of selection which could influence a locus.

Seeds

Seed size (and shape) vary among different plant species, ranging from extremely small (2 µg in the orchid, *Goodyera repens*) to enormous (27 kg, seed of the double coconut palm, *Lodoicea maldivica*; Harper *et al*. 1970). Seed size is presumably relevant to seed dispersal, longevity and seedling establishment. Population diversity may emerge in an area as a result of germination of seeds that have persisted in soil for years (Olatunde Akinola *et al*. 1998; Roberts 1968) or due to migration involving various dispersal agents, such as wind or animals (clinging to skin or fur, consumption and subsequent excretion etc). As an example of the latter, seeds of *Arabidopsis thaliana* were reported to germinate from rabbit dung (Malo 1995)

Volis, Mendlinger and Ward (2002) examined local adaptation of wild barley in a gradient climate from four different origins: desert, Mediterranean semi-steppe batha (open vegetative community dominated by shrubs), Mediterranean grassland, and mountainous. In two years of reciprocal transplant experiments, they found evidence for local adaptation involving several traits. Each genotype produced the highest seed yield in their native environment; the lowest fitness (seed yield) was seen in reciprocal transplants of desert and mountain genotypes (i.e. switched from one native extreme habitat condition to an opposite extreme). Both Mediterranean plants also had their maximum fitness in grassland and mountain (higher climate predictability), with decreased fitness through semi-steppe batha and desert (increasing unpredictability). In particular, both Mediterranean genotypes yielded fewer but larger seeds than desert plants

in semi-steppe batha, grassland and desert sites, where desert plants grew more but smaller seeds at all sites except mountain. Both seed production strategies are consistent adaptations to specific local climate conditions. Volis *et al*. also found that larger seeds had higher seedling vigour whereas a greater number of smaller seeds could be a "bet-hedging" strategy - a feature of drought avoidance, where seeds delayed germination in an unpredictable environment, with the result of increasing the chances of producing mature plants. Their findings illustrated the dynamic of the seed size relationship across a gradient environment and emphasized the importance of seed size within a plant's reproductive success.

A. thaliana accessions from different regions have been found to differ in seed mass. For example, the L*er* produced twice as many seeds as *Cvi*, but its seeds were around 50% lighter (Alonso-Blanco *et al*. 1999). QTL underlying this variation were mapped and found to act either in the mother plant or in the seeds themselves. There is as yet no evidence that this variation in seed size is adaptive in *A. thaliana*.

Aims and objectives

This chapter will focus on growth variation under natural conditions of wild *A. thaliana* genotypes gathered from the Edinburgh area. The plants were grown in semi-natural conditions in the field, to expose plants to the numerous aspects of variation within the natural environment that were evidenced by the weather data in Chapter 4.

The motivation for this work was to test whether populations of *A. thaliana* might be adapted to their local environments. Adaptive evolution demands that there is variation in phenotype (in this case growth), which is genetically determined (i.e., has high heritability) and produces a fitness differential. Over time, this process can change the allele frequency of a given population. It was therefore tested whether different populations of *A. thaliana* showed heritable differences in growth rate, and whether different families performed better in different environments. As part of this process, an

intention was taken to attempt to isolate likely environmental factors affecting the fitness of genotypes differently.

Both flowering time and seed yield were also examined. This was performed with an aim to better understand the relationship between growth rate and reproduction of local populations under the influence of natural conditions. Seed yield of surviving plants offered an insight into the relationships between growth rate and fitness, estimated from seed yield – whether greater speed of growth is a beneficial strategy under all conditions.

Results

The results in this chapter are divided into three main sections: 1) Relative growth rate and seasonal growth performance of local *Arabidopsis thaliana* populations, 2) flowering time variation and 3) fitness estimated from seed yield. The first section of the chapter includes an explanation and justification of the methodology for estimating growth rate, as well as the result of growth rate heritability estimates and relationships between growth rates at different sites and in different seasons.

Significant differences were found in the growth rate(s) of the local population under study. The following sections discuss this variation and its relationship to growth conditions at each of the three experiment sites. Understanding where and when a correlation between growth and particular climate conditions occurs, offers an insight into the environmental factors that might be involved in local adaptation.

5-1 Seasonal Growth

Experimental design

As *Arabidopsis thaliana* can be winter (germinating in autumn and flowering the following spring) or summer annual (germinating in spring and flowering the same year), autumn and spring field experiments were performed to examine the impact of seasonal difference upon growth performance.

Consequently, plants were grown in six seasons, with germination either in September or March, from September 2007 to July 2010. Five sets of seasonal experiments examined growth, flowering and seed yields of plants growing in isolation and the last two also examined the effects of competition between accessions.

The experiments investigated the effects of geographical elevation: involving field sites at 67 m (low elevation) and 300 m (high elevation), which reflected the extremes of elevation of the original sampling sites. Plants were grown at each of these sites in the six seasonal experiments. A subset of plants was grown in an unheated, unlit greenhouse, to serve as a test site with more mediated climate conditions than the exposed outdoor sites.

Because families differed in seed dormancy, germination was synchronized to correspond to germination of natural seedlings in the field. In the initial experiments (autumn 2007), seeds were treated with gibberellic acid (GA) to induce germination. Once the time taken for each accession to germinate without GA had been determined, GA treatment was discontinued and sowing were staggered, when necessary for synchronous germination.

Seedlings were germinated at 20°C in 16 hour light, approximating to the natural daily dark-light cycle. They were transplanted five days after germination and transferred to the field sites after an additional three to five days in an unheated greenhouse. Seedlings

of similar size were selected to minimize any effects of different germination times. Day length was approximately 12 hours in the field during the first week whether the seedlings were grown in September (autumn) or March (spring).

Growth rate was estimated from rosette areas, measured from digital images taken at weekly intervals when growth was relatively slow during autumn or winter and twice weekly in during periods of more rapid growth in spring.

This photographic method of estimating rosette area provides advantages over traditional destructive methods since it is neither invasive nor destructive. More importantly, the same plant can be monitored throughout the entire life cycle, instead of using different replicates of individuals from the same family. This minimizes the contribution of variance within families to the errors in estimating growth rates. Additionally, previous work has shown that rosette area was a good proxy for mass, for it was directly proportional to fresh weight, with an r^2 value of 0.89 (Fig. 5-1-1).

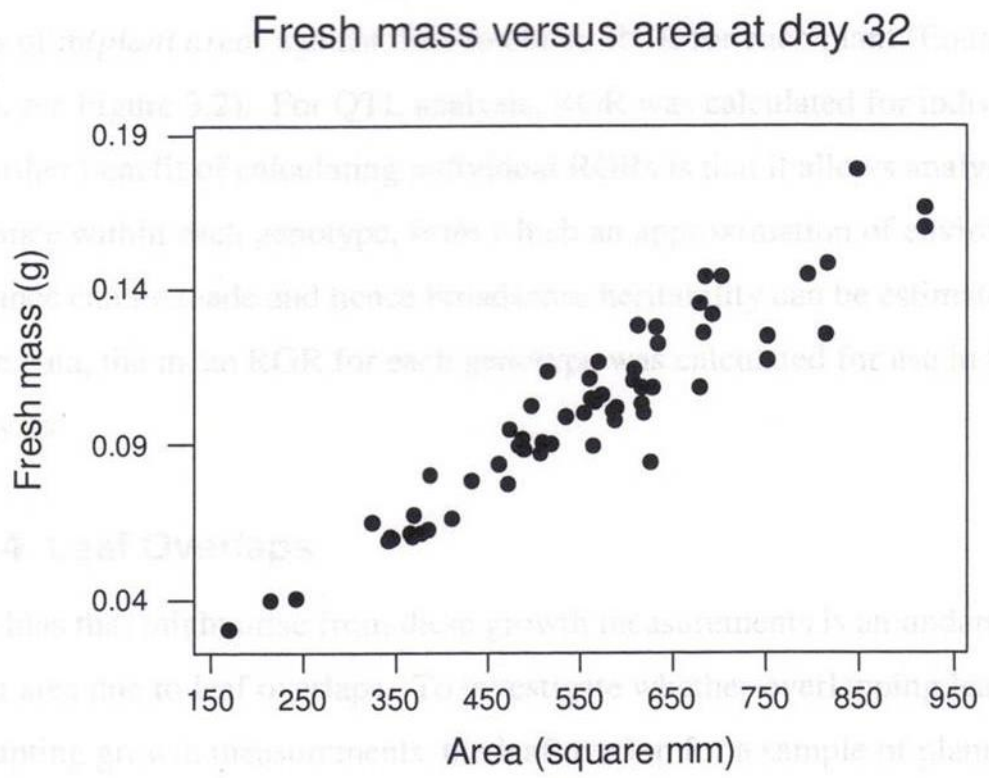

Figure 5-1-1 **Regression of fresh mass against plant rosette area at 32 days, as recorded by J. Atkinson**. Rosette area is well correlated with fresh mass - the coefficient of determination, r^2 is 0.89. For dry mass against rosette area, r^2 = 0.96. Total plant number = 119 (J. Atkinson, PhD thesis 2006).

Using rosette area as a proxy for growth rate

For the reason that leaf growth can approach an exponential rate, growth is often estimated as relative growth rate (RGR) - the rate of increase in area per unit area, most commonly calculated as the rate of change in the natural log of area: (lnA_f – lnA_i) / T, where A_f is final area measurement, A_i is the initial are measurement and T is time in days

However, it is important to acknowledge a potential disadvantage of RGR; it involves at least two estimates of area, each with an associated error (Hoffman & Poorter 2002).

This could potentially lead to a less accurate estimate of genetically determined growth rate than an estimate made from a single measurement.

Initial growth chamber experiments – in long day (LD; 16 hours light) or short day (SD; 8 hours light) – were therefore used to assess different methods of estimating growth rate. The average rosette area of all accessions, grown in controlled conditions of 16°C SD, increased at a rate that was faster than linear (Fig. 5-1-2) so the differences in rosette area between accessions became more obvious with time. Earlier growth rates in the experiment were largely related to those later in the experiment, for example the rate of increase in area of family 5A3 (shown by the slope of the red line in Fig. 5-1-2A) was the highest throughout the experiment and family 12A1 (the purple line in Fig. 5-1-2A) showed the lowest rate of increase up to about 38 dsg (days since germination).

However, the later rate of area increase does not reflect earlier rates for some families. For example, the rosettes of family 10B1 are smaller than 2E5 at 40 dsg, but significantly larger at 45 dsg. It is possible that the altered growth rates reflect developmental changes, such as the transition to bolting (i.e. 2E5 is an earlier bolting genotype than 10B1) or differences in the degree of leaf overlap in different families. It was difficult to determine the impact of these factors upon the rosette area measurements. Hence, it was desirable to measure rosette area at an earlier stage when such developmental changes were less likely to affect growth rate estimates and when leaf overlap would be minimal. However, it is also necessary to select a time point sufficiently late for the plants to have established themselves and to have grown to a size that allowed more accurate estimates of rosette area. It was also observed that younger plants had fewer overlapping leaves and more consistent leaf shapes compared to older plants of around 56 dgs (Fig. 5-1-2B). Hence, rosette area measurements were taken between 24 and 45 dsg.

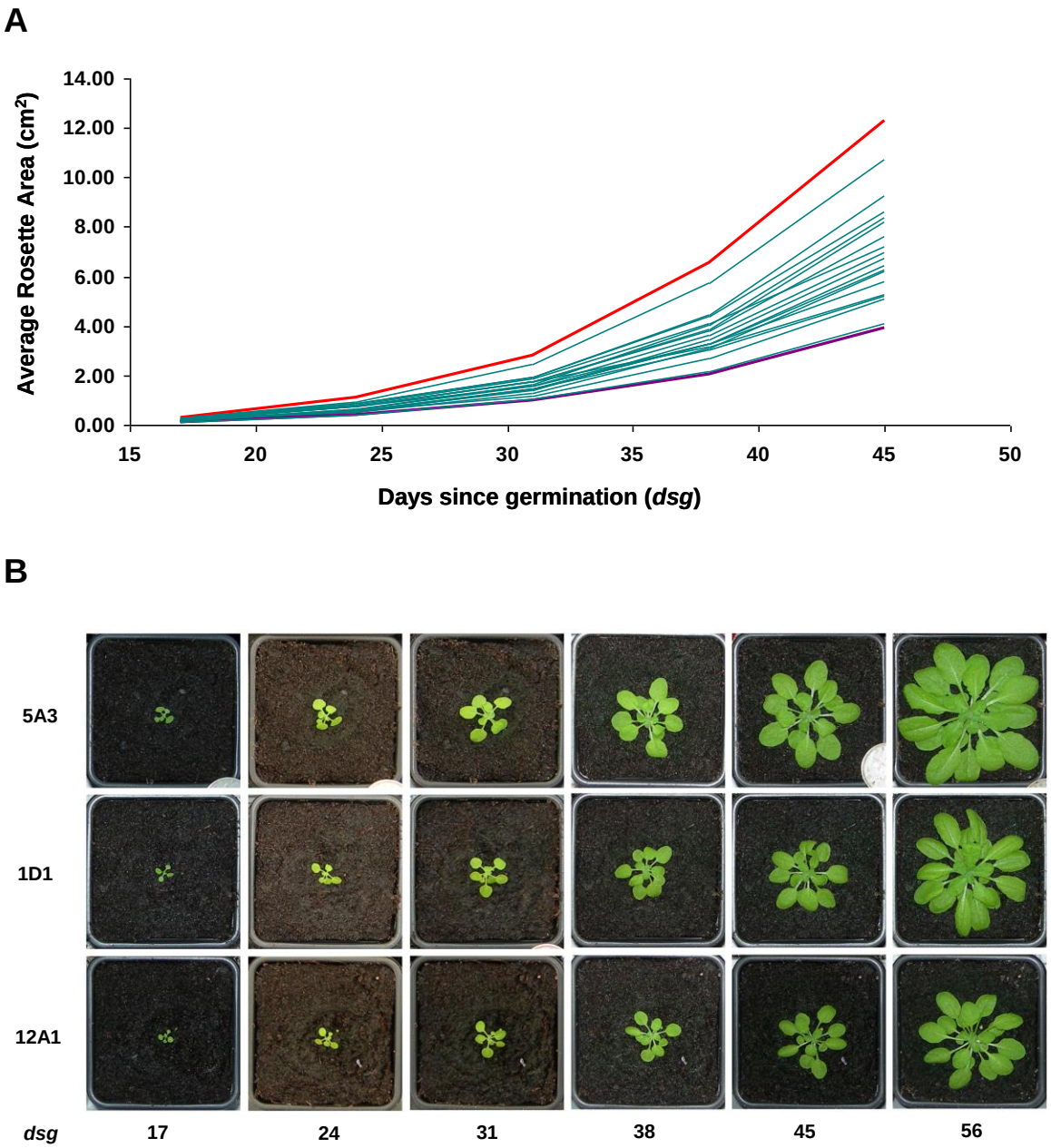

Figure 5-1-2 **Growth in rosette area**. The average rosette area (cm^2) of all families from 17 days since germination (dsg) to 45 (dsg) in a controlled environment (16°C, SD) is shown (**A**). For clarity, variance within families is not shown. The fastest growing genotype, family 5A3, is shown in red and the slowest, 12A1, in purple. Examples of the fastest growing family, 5A3, intermediate family 1D1, and the slowest growing, 12A1 are shown (**B**). Leaf areas show more than a linear rate of increase, resulting a greater difference in total rosette areas between the fastest and slowest accessions later in the experiment.

RGR Heritability

Broad-sense heritability, H^2, is defined as the amount of phenotypic variation which can be attributed to genetic variation: this includes any genetic contribution such as epistatic interactions. H^2 was estimated by comparing the variation within families (which has a non-genetic basis) to the variation between families, which is due to both genetic and non-genetic factors.

H^2 values for RGR were high but varied throughout an experiment – for example, at LE in autumn 2009 they varied from 0.19 to 0.89. They also tended to be lower than H^2 for rosette area.

RGR might have lower heritability than a single measurement of rosette area firstly because of the effect of compound errors in RGR calculation and secondly because the environment might change between time-points and affect plant differently. Therefore only single area measurements were used as an estimate of growth rate in this study.

Heritable differences in growth rate from seasonal experiments

Rosette area measurements (taken between 31 to 146 dsg) were used to estimate heritability in 14 seasonal experiments, each involving between six and twelve members of each family (genotype). The values were found to range between 0.32 to 0.91, with the majority over 0.60 (Table 5-1-1A). There was no significant difference in the heritability values for the different sites, or between autumn and spring experiments.

LE and HE growth (rosette area) H^2 was also estimated within individual sample group (families that collected from the same location) from autumn 2007 and spring 2008 (Table 5-1-1B). Some growth differences were observed to be significant and highly heritable within sample group. For example, H^2 estimates for most autumn growth at either or, both LE and HE were observed to be very high in sample groups contained

families that did not cluster as a clade on the local phylogenetic tree (i.e. sample group 1, 5 and 6) (Chapter 3). Insignificant growth differences between families, as well as low H^2 were observed in most groups that consist of families that were clustered within a clade (i.e. 10 and 11). In spring, growth H^2 was low for most sample groups at LE and/ or HE.

Furthermore, growth data from autumn 2007 and spring 2008 that contained at least five families per sample group were tested with nested-ANOVA (Table 5-1-1C). The variability attributable to sites, families within sites and within families of the total variability, varies among sample groups and between seasons. In general, the variability attributable to sites and families within sites was low in both seasons.

Consistently high H^2 values for vegetative growth rate have not been reported previously. It suggests that local accessions show genetically determined differences in growth rate under field conditions. This raises a question of why genetic variation for growth rate is maintained in local populations. One possibility is that the differences reflect adaptations to the environments from which the genotypes were collected.

	2007	**2008**		**2009**	
	Autumn	**Spring**	**Autumn**	**Spring**	**Autumn**
LE	0.80	0.57	0.68	0.74	0.87
HE	0.76	0.49	0.48	0.65	0.89
GH	0.32	0.69	0.91	0.72	-

Table 5-1-1A **Summary of heritability (H^2) estimated from rosette area**. Local *A. thaliana* populations grown in all seasonal experiments displayed remarkably high heritability. Keys: **LE**, low elevation; **HE**, high elevation and **GH**, unheated unlit greenhouse.

Autumn 2007	LE						HE					
Sample Group 1	**SS**	**df**	**MS**	**Fs**	**p**	**Var comp (%)**	**SS**	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Between families	5.7095	3	1.9032	12.8083	0.00013	69.40	6.3784	3	2.1261	11.9229	0.0001	65.56
Within families	2.5260	17	0.1486			30.60	3.3881	19	0.1783			34.44
Total	8.2354	20					9.7665	22				
Sample Group 2												
Between families	10.6774	5	2.1355	16.0348	1.8E-07	72.65	8.5474	5	1.7095	7.0087	0.0002	50.76
Within families	3.7290	28	0.1332			27.35	7.0734	29	0.2439			49.24
Total	14.4064	33					15.6208	34				
Sample Group 4												
Between families	0.8579	1	0.8579	3.8232	0.0791	-	3.7592	1	3.7592	14.1345	0.0045	70.66
Within families	2.2438	10	0.2244			-	2.3936	9	0.2660			29.34
Total	3.1017	11					6.1528	10				
Sample Group 5												
Between families	35.1026	3	11.7009	91.3988	1.1E-10	94.55	21.0943	3	7.0314	23.5312	1.3E-06	79.70
Within families	2.1763	17	0.1280			5.45	5.6775	19	0.2988			20.30
Total	37.2789	20					26.7718	22				
Sample Group 6												
Between families	7.5860	3	2.5287	10.8935	0.00016	61.32	31.2518	3	10.4173	38.5010	8.5E-08	87.74
Within families	4.8746	21	0.2321			38.68	4.5997	17	0.2706			12.26
Total	12.4606	24					35.8515	20				
Sample Group 7												
Between families	0.4325	1	0.4325	4.2448	0.0664	-	0.2022	1	0.2022	0.9983	0.3413	-
Within families	1.0189	10	0.1019			-	2.0252	10	0.2025			-
Total	1.4513	11					2.2274	11				
Sample Group 8												
Between families	0.0728	1	0.0728	1.6678	0.2326	-	0.1462	1	0.1462	1.1279	0.3159	-
Within families	0.3491	8	0.0436			-	1.1669	9	0.1297			-
Total	0.4219	9					1.3131	10				

Table 5-1-1B **H^2 of individual sample group estimated from LE and HE growth (rosette area) in autumn 2007 and spring 2008 (1 of 4)**.

Sample Group 9												
Between families	2.3305	2	1.1653	8.5958	0.0033	55.87	0.1894	2	0.0947	0.8873	0.4323	-
Within families	2.0334	15	0.1356			44.13	1.6007	15	0.1067			-
Total	4.3639	17					1.7901	17				
Sample Group 10												
Between families	0.6232	1	0.6232	3.5328	0.0929	-	4.5902	1	4.5902	5.5934	0.0396	43.36
Within families	1.5876	9	0.1764			-	8.2065	10	0.8207			56.64
Total	2.2108	10					12.7967	11				
Sample Group 11												
Between families	6.7629	4	1.6907	4.2484	0.0107	37.69	21.4304	4	5.3576	8.0221	0.0003	53.92
Within families	8.7553	22	0.3980			62.31	16.6964	25	0.6679			46.08
Total	15.5182	26					38.1268	29				
Sample Group 13												
Between families	17.9160	3	5.9720	28.5831	4.6E-07	83.41	3.0427	3	1.0142	3.8491	0.0262	33.17
Within families	3.7608	18	0.2089			16.59	5.0064	19	0.2635			66.83
Total	21.6768	21					8.0491	22				

Table 5-1-1B **H^2 of individual sample group estimated from LE and HE growth (rosette area) in autumn 2007 and spring 2008 (2 of 4)**.

Spring 2008	LE						HE					
Sample Group 1	**SS**	**df**	**MS**	**Fs**	**p**	**Var comp (%)**	**SS**	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Between families	13.5712	9	1.5079	17.9527	1.3E-14	69.10	15.1817	9	1.6869	6.1128	3.1E-06	39.94
Within families	5.5436	66	0.0840			30.90	18.4890	67	0.2760			60.06
Total	19.1148	75					33.6707	76				
Sample Group 2												
Between families	5.6339	10	0.5634	6.8397	2.2E-07	43.95	10.9404	10	1.0940	7.6364	4.5E-08	47.77
Within families	5.8483	71	0.0824			56.05	9.8853	69	0.1433			52.23
Total	11.4822	81					20.8258	79				
Sample Group 4												
Between families	2.4063	6	0.4010	3.8410	0.0041	29.77	1.4436	6	0.2406	1.2976	0.2799	-
Within families	4.1764	40	0.1044			70.23	7.6026	41	0.1854			-
Total	6.5827	46					9.0463	47				
Sample Group 5												
Between families	1.5551	3	0.5184	5.9663	0.0031	39.87	3.2527	3	1.0842	4.1024	0.0153	27.35
Within families	2.2590	26	0.0869			60.13	7.6645	29	0.2643			72.65
Total	3.8142	29					10.9172	32				
Sample Group 6												
Between families	2.3933	4	0.5983	9.6728	2.7E-05	53.32	7.9571	4	1.9893	11.4170	9.5E-06	59.86
Within families	2.0413	33	0.0619			46.68	5.2272	30	0.1742			40.14
Total	4.4346	37					13.1843	34				
Sample Group 7												
Between families	1.0489	1	1.0489	9.4428	0.0083	51.35	1.5880	1	1.5880	12.2016	0.0050	63.42
Within families	1.5551	14	0.1111			48.65	1.4316	11	0.1301			36.58
Total	2.6040	15					3.0196	12				
Sample Group 8												
Between families	9.5248	4	2.3812	12.9041	2.0E-06	61.10	13.7528	4	3.4382	19.0192	1.2E-07	73.40
Within families	6.0895	33	0.1845			38.90	5.0617	28	0.1808			26.60
Total	15.6143	37					18.8145	32				

Table 5-1-1B **H^2 of individual sample group estimated from LE and HE growth (rosette area) in autumn 2007 and spring 2008 (3 of 4)**.

Sample Group 9												
Between families	0.2287	2	0.1144	1.7132	0.2057	-	0.8141	2	0.4070	1.9923	0.1670	-
Within families	1.3350	20	0.0668			-	3.4731	17	0.2043			-
Total	1.5638	22					4.2872	19				
Sample Group 10												
Between families	1.4235	5	0.2847	1.3474	0.2670	-	2.0459	4	0.5115	1.8526	0.1423	-
Within families	7.6066	36	0.2113			-	9.1108	33	0.2761			-
Total	9.0301	41					11.1567	37				
Sample Group 11												
Between families	0.4327	6	0.0721	1.2664	0.2897	-	0.9932	6	0.1655	1.1096	0.3727	-
Within families	2.8473	50	0.0569			-	6.4151	43	0.1492			-
Total	3.2799	56					7.4083	49				
Sample Group 12												
Between families	0.3957	1	0.3957	5.0438	0.0392	31.00	2.0163	1	2.0163	37.9429	1.8E-05	81.35
Within families	1.2554	16	0.0785			69.00	0.7971	15	0.0531			18.65
Total	1.6511	17					2.8134	16				
Sample Group 13												
Between families	1.2543	6	0.2091	3.1779	0.0118	24.17	0.9890	6	0.1648	1.1726	0.3392	-
Within families	2.6971	41	0.0658			75.83	5.9041	42	0.1406			-
Total	3.9515	47					6.8931	48				

Table 5-1-1B **H^2 of individual sample group estimated from LE and HE growth (rosette area) in autumn 2007 and spring 2008 (4 of 4)**. Some growth differences were observed to be significant and highly heritable within sample group, whereas others were not. Key: **Sample group #** (plants sampled from location #, i.e. 1-13); Key: **LE**, low elevation; **HE**, high elevation; **SS**, sum of squares; **df**, degree of freedom; **MS**, mean squares; **Fs**, F-ratio; **Var comp**, variance component.

Autumn 2007						
Sample Group 2	**SS**	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sites (LE vs HE)	10.4086	1	10.4086	5.4141	0.042278	33.34
Families within sites	19.2248	10	1.9225	10.1442	1.55E-09	40.94
Within families	10.8023	57	0.1895			25.72
Total	40.4358	68				100.00
Sample Group 11						
Among sites (LE vs HE)	22.3105	1	22.3105	6.3307	0.036	38.23
Families within sites	28.1933	8	3.5242	6.5078	1.1E-05	30.40
Within families	25.4517	47	0.5415			31.37
Total	75.95551	56				100.00

Table 5-1-1C **Nested ANOVA for growth of various sample groups at LE and HE in autumn 2007 and spring 2008 (1 of 2)**.

Spring 2008						
Sample Group 1						
Among sites (LE vs HE)	2.8751	1	2.8751	1.7999	0.19641	4.27
Families within sites	28.7529	18	1.5974	8.8402	2.59E-15	48.50
Within families	24.0326	133	0.1807			47.23
Total	55.6606	152				100.00
Sample Group 2						
Among sites (LE vs HE)	2.8911	1	2.8911	3.3143	0.0845	10.77
Families within sites	16.5739	19	0.8723	7.7702	6.10E-14	42.87
Within families	14.9310	133	0.1123			46.36
Total	34.3960	153				100.00
Sample Group 4						
Among sites (LE vs HE)	0.2357	1	0.2357	0.7347	0.408153	0.00
Families within sites	3.8499	12	0.3208	2.2062	0.018695	15.13
Within families	11.7790	81	0.1454			84.87
Total	15.8647	94				100.00
Sample Group 6						
Among sites (LE vs HE)	5.4987	1	5.4987	4.2500	0.073184	29.37
Families within sites	10.3505	8	1.2938	11.2142	1.04E-09	41.22
Within families	7.2685	63	0.1154			29.41
Total	23.1176	72				100.00
Sample Group 8						
Among sites (LE vs HE)	0.2696	1	0.2696	0.0927	0.768593	0.00
Families within sites	23.2776	8	2.9097	15.9168	2.16E-12	67.89
Within families	11.1512	61	0.1828			32.11
Total	34.6984	70				100.00
Sample Group 10						
Among sites (LE vs HE)	0.0725	1	0.0725	0.1881	0.674683	0.00
Families within sites	3.4694	9	0.3855	1.5911	0.135204	7.55
Within families	16.7174	69	0.2423			92.45
Total	20.2594	79				100.00
Sample Group 11						
Among sites (LE vs HE)	0.8275	1	0.8275	6.9642	0.021615	11.52
Families within sites	1.4259	12	0.1188	1.1931	0.29974	2.19
Within families	9.2623	93	0.0996			86.29
Total	11.5157	106				100.00
Sample Group 13						
Among sites (LE vs HE)	0.6044	1	0.6044	3.2332	0.097346	6.89
Families within sites	2.2433	12	0.1869	1.8039	0.060631	9.71
Within families	8.6013	83	0.1036			83.40
Total	11.4490	96				100.00

Table 5-1-1C **Nested-ANOVA for growth of various sample groups at LE and HE in autumn 2007 and spring 2008 (2 of 2)**. Autumn and spring growth, where at least five families per sample group were planted per experiment, were tested with nested-ANOVA. Key: **Sample group #** (plants sampled from location #, i.e. 1-13); Key: **LE**, low elevation; **HE**, high elevation; **SS**, sum of squares; **df**, degree of freedom; **MS**, mean squares; **Fs**, F-ratio; **Var comp**, variance component.

The effects of site and season on growth rate

Having shown that variation in growth rate was highly heritable in most experiments, the average growth rate of all accessions was compared between experiments. Nested-ANOVA was performed within each experiment. The variability attributable to among sample groups, families within group and within families, varies among experiments (Table 5-1-2).

In all years and seasons, growth in greenhouse conditions (GH) was faster than at low elevation (LE). For example, in autumn 2007, it took plants at LE 11 days more to reach the size of GH plants at 31 dsg. By 42 dsg, GH plants had a mean rosette area of 9.8 cm^2; three times larger than the LE and HE cohorts of the same age. Similarly, but with even greater contrast, GH plants were 5.0 cm^2 at 31 dsg and reached 24.1 cm^2 at 42 dsg in spring 2008; whereas the means of the LE and HE were approximately 2 cm^2 – one twelfth that of GH (Table 5-1-2).

In general, plants at LE grew faster than at HE in all seasons (based on the population mean), with the sole exception of autumn 2007 (Table 5-1-2). The population mean of LE plants in autumn 2007 was 2.8 cm^2 at 42 dsg, nearly 1 cm^2 smaller than at HE. Nonetheless, LE plants grew faster than HE plants from spring 2008 onwards, particularly in spring and autumn 2009. The population mean rosette area of spring LE in 2008 (1.9 cm^2 at 49 dsg) was nearly the same as HE seven days later (2.1 cm^2 at 56 dsg). Furthermore, the population means of LE plants in both spring and autumn 2009 (at 39 dsg) were over 0.5 cm^2 larger than those grown at HE (Table 5-1-2). This suggests that the environmental differences between experimental sites had an effect on growth rate. Vegetative growth variation was unsurprisingly different between seasons. GH plants for example, always grew faster in spring than in autumn. The population mean in autumn 2007 was 2.8 cm^2 at 31 dsg, but the mean of the spring cohorts in 2008 was 5.0 cm^2 at the same age. The population mean of GH in autumn 2008 was again half of spring 2009

(1.4 cm^2 at 32 dsg and 3.2 cm^2 at 33 dsg respectively), even though the spring 2009 set was just one day older (Table 5-1-2).

Plants at LE and HE, however, tended to grow faster in autumn than in spring: Table 5-1-2 shows that LE and HE plants grew faster in two of out of three autumns (2007 and 2009) compared to spring. These autumn LE and HE plants were between 1.0 cm^2 to 2.5 cm^2 larger on average than those grown in spring 2008 and 2009.

Interestingly, both the fastest and slowest recorded field growth was observed in autumn seasons. The fastest LE and HE growth was recorded in autumn 2009 (5.7 cm^2 and 4.9 cm^2 respectively at 39 dsg) and the slowest in autumn 2008, when the LE population mean was only 1.3 cm^2 at 46 dsg (data not shown).

LE						
Autumn 2007	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	220	39	42	2.8	0.06	0.93
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	79.2104	11	7.2009	2.2076	0.045963	22.25
Families within groups	88.0711	27	3.2619	17.4588	3.01E-37	58.09
Within families	33.8168	181	0.1868			19.66
Total	201.0983	219				100.00
Autumn 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	237	18	144	4.4	0.11	1.96
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	21.6570	10	2.1657	6.6462	0.009887	56.04
Families within groups	2.2810	7	0.3259	6.6237	4.12E-07	12.85
Within families	10.7738	219	0.0492			31.11
Total	34.7118	236				100.00
Autumn 2009	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	176	20	39	5.7	0.14	3.64
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	484.9424	11	44.0857	5.0144	0.015178	63.42
Families within groups	70.3347	8	8.7918	18.0541	6.22E-19	24.05
Within families	75.9675	156	0.4870			12.53
Total	631.2446	175				100.00
Spring 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	516	69	49	1.9	0.02	0.23
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	31.4043	11	2.8549	4.0840	0.000199	22.16
Families within groups	39.8461	57	0.6991	7.2142	3.45E-36	35.44
Within families	43.3141	447	0.0969			42.40
Total	114.5646	515				100.00
Spring 2009	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	134	19	39	3.3	0.08	0.93
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	84.5105	11	7.6828	8.5395	0.004539	65.57
Families within groups	6.2977	7	0.8997	3.9823	0.000621	10.42
Within families	25.9803	115	0.2259			24.01
Total	116.7885	133				100.00

Table 5-1-2 **Population mean rosette area (cm^2) in different seasons with nested-ANOVA (1 of 3)**.

HE						
Autumn 2007	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	225	39	42	3.6	0.07	1.32
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	114.8412	11	10.4401	2.8011	0.014299	28.59
Families within groups	100.6321	27	3.7271	11.8579	7.31E-28	46.67
Within families	58.4628	186	0.3143			24.74
Total	273.9361	224				100.00
Autumn 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	185	16	146	3.1	0.08	1.15
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	67.0468	9	7.4496	1.3810	0.358319	11.19
Families within groups	32.3674	6	5.3946	9.4435	6.13E-09	37.01
Within families	96.5411	169	0.5712			51.80
Total	195.9553	184				100.00
Autumn 2009	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	181	20	39	4.9	0.16	4.88
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	665.4530	11	60.4957	3.8104	0.034039	56.11
Families within groups	127.0107	8	15.8763	27.9180	7.16E-27	33.10
Within families	91.5569	161	0.5687			10.79
Total	884.0206	180				100.00
Spring 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	494	67	56	2.1	0.03	0.39
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	40.1840	11	3.6531	3.3311	0.001411	16.60
Families within groups	61.4125	56	1.0967	5.7110	8.18E-27	32.87
Within families	81.8024	426	0.1920			50.53
Total	183.3990	493				100.00
Spring 2009	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	153	19	40	2.6	0.07	0.74
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	67.2726	11	6.1157	4.9496	0.021854	49.02
Families within groups	8.6491	7	1.2356	4.5995	0.000123	16.41
Within families	35.9969	134	0.2686			34.57
Total	111.9186	152				100.00

Table 5-1-2 **Population mean rosette area (cm^2) in different seasons with nested-ANOVA (2 of 3)**.

GH						
Autumn 2007	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	56	19	31	2.8	0.17	1.73
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	650.067	10	65.0067	1.2889	0.366698	12.75
Families within groups	403.474	8	50.4343	16.6492	3.93E-10	73.51
Within families	112.082	37	3.0292			13.74
Total	1165.622	55				100.00
Autumn 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	117	20	32	1.4	0.04	0.20
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	254.822	11	23.1656	20.4748	0.000116	85.71
Families within groups	9.051	8	1.1314	5.0017	3.3E-05	5.87
Within families	21.942	97	0.2262			8.43
Total	285.815	116				100.00
Spring 2008	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	69	23	31	5.0	0.16	1.80
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	10.615	11	0.9650	0.9953	0.503073	0.00
Families within groups	10.665	11	0.9696	7.6578	2.67E-07	68.94
Within families	5.824	46	0.1266			31.06
Total	27.104	68				100.00
Spring 2009	**n**	**n fam**	**dsg**	**Pop mean**	**SE**	**Var**
	103	19	33	3.2	0.08	0.59
	SS	**df**	**MS**	**Fs**	**p**	**Var comp (%)**
Among sample groups	37.914	11	3.4467	3.1340	0.070107	43.45
Families within groups	7.698	7	1.0998	6.5024	3.93E-06	29.33
Within families	14.207	84	0.1691			27.23
Total	59.819	102				100.00

Table 5-1-2 **Population mean rosette area (cm^2) in different seasons with nested-ANOVA (3 of 3)**. Plants in GH grew faster than at LE and HE in the same season. Key: **LE**, low elevation; **HE**, high elevation; **GH**, unheated unlit greenhouse; **n**, population size; **n fam**, number of families; **dsg**, days since germination; **Pop mean**, population mean area (cm^2); **SE**, population standard error; **Var**, population variance; **SS**, sum of squares; **df**, degree of freedom; **MS**, mean squares; **Fs**, F-ratio; **Var comp**, variance component; **sample groups**, families from the same sampling location - i.e.1 to 13.

Effects of elevation on relative performances

High genetically determined differences in growth between accessions suggested the possibility that the local accessions were adapted to the different environments from which they were originally sampled. The accessions were collected from different elevations. Since elevation has an effect on growth rate (Table 5-1-2), one possibility

was that the accessions were adapted to environmental factors that vary with elevation. This was investigated by comparing the growth rates of accessions at LE and HE in the same season (Fig. 5-1-3A).

To allow comparisons between experiments, which differed in the mean growth rate of their members, growth rates within each experiment were estimated from rosette areas and normalized (by subtracting the mean for the experiment and dividing by the standard deviation). The normalized value for each accession in an experiment is subsequently referred to as its relative performance.

The growth rates of accessions at LE and HE were found to be strongly correlated ($r = 0.71$, $p = 0.001$): those that grew faster at HE also tended to grow faster at LE (Figure 5-1-3A) and vice versa. This suggests that the accessions are not adapted to environmental factors that vary between elevations in the same season; otherwise some would be expected to perform relatively better at LE and some at HE.

However, comparing the relative performance of accessions between seasons revealed a lack of correlation ($r = 0.14$, $p = 0.24$; Fig. 5-1-3B). Some accessions that performed well in autumn were observed to grow more poorly in spring, while other accessions performed better in spring than autumn. This data suggests that accessions may be adapted to environmental factors that vary between seasons, but not with between elevations in the same season.

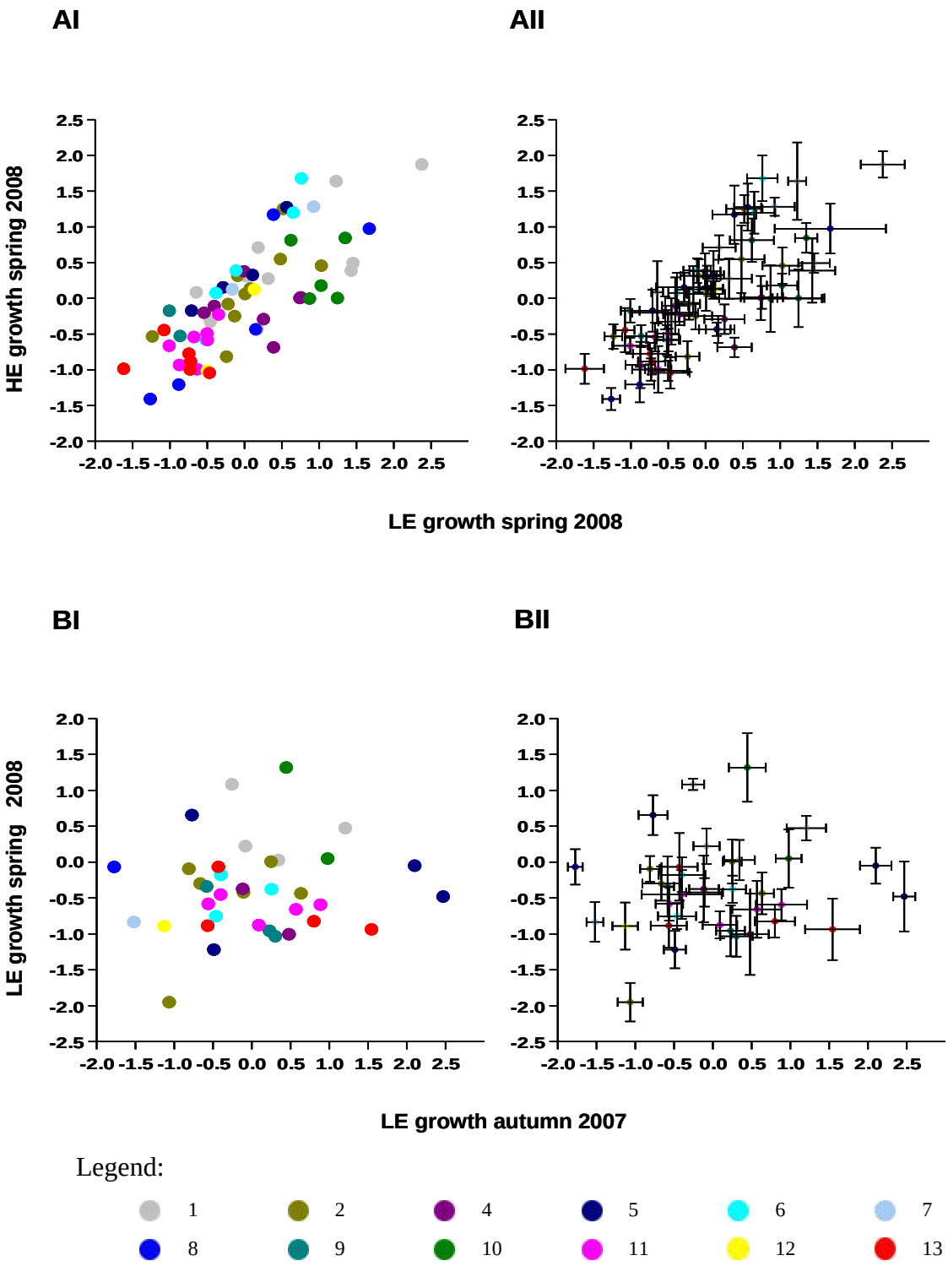

Figure 5-1-3 **Regression of growth at different elevations (LE and HE) and seasons (autumn and spring) with SE**. Growth rates at LE and HE within the same spring season are strongly correlated (r = 0.71, p = 0.001) (**A**). 5-1-3**B** shows no significant correlation between growth of accessions at LE growth between autumn

2007 and spring 2008 (r = 0.14, p = 0.24). Values are means (of normalized values) for an average of five plants for each accession and bars show standard errors. Plant families are colour coded based upon the sampling locations (1 to 13; refer maps in Chapter 2) for the local accessions (**I**) with ± *SE* (**II**).

Effects of season on relative performances

In each year, local *A. thaliana* accessions were found to be generally slower growth (smaller rosette areas) at HE than at LE; this held for both autumn and spring. The relative growth performances of these accessions within the same season were often found to be highly correlated between both sites. This was particularly true for autumn. For example, the growth of LE and HE cohorts were highly correlated in autumn 2007 and 2008 (r = 0.72, p = 0.0001 and r = 0.71, p = 0.002). The strongest correlation between LE and HE was recorded in autumn 2009, where r = 0.90 (p = 0.0001) (Table 5-1-3).

Most correlations were reduced or became insignificant between different autumns; this applies both when comparing the same site in different years and when comparing different sites in different years. For example, growth at LE site between autumn 2007 and 2008 was weakly correlated (r = 0.34, p = 0.21), and growth between autumn 2008 LE cohorts and autumn 2009 HE cohorts was 0.66 (p = 0.004) (Table 5-1-3). This reduction is consistent with annual seasonal weather variation.

As in autumn, growth of spring plants at different elevations was correlated strongly within the same year: with r-values equal to 0.71 (p = 0.001) and 0.70 (p < 0.001) for spring 2008 and 2009 respectively. In addition, the growth of accessions showed higher correlations between springs of different years that they had for autumns. Growth at LE in spring 2008 was significantly well correlated to that in spring 2009 (r = 0.60, p = 0.008), as was growth at HE site in these two different springs (r = 0.56, p = 0.02) (Table 5-1-3).

Growth in autumn was only poorly correlated with growth in spring, For example, the growth correlation of LE cohorts between autumn 2007 and spring 2008 was insignificant ($r = 0.14$, $p = 0.24$). This implies accessions that grew well in autumn did not necessarily grow well in spring, and vice versa (Table 5-1-3).

There was a consistent lack of growth correlation between autumn and spring seasons. There was a total of 24 pair of autumn and spring combinations, most severely lacking correlation with one exception: growth at LE autumn 2008 and HE spring 2009 ($r = 0.70$, $p = 0.001$), where autumn and spring growth correlated as well as the growth correlation between elevations in either autumn or spring in the same years. This may be attributable to a similarity in seasonal elements experienced at these locations and time periods.

In summary, field grown accessions showed strong growth correlations between elevations within a season but not between autumn and spring. This is consistent with an adaptation to season, rather than elevation. The observed correlation between the same seasons in different years suggests a predictable variation in environmental variables, influencing the growth of these local accessions accordingly.

A

		LE					HE				
		A 2007	**A 2008**	**A 2009**	**Sp 2008**	**Sp 2009**	**A 2007**	**A 2008**	**A 2009**	**Sp 2008**	**Sp 2009**
LE	**A 2007**	-	0.34	0.31	0.14	0.03	0.72 $^{+}$*	0.74	0.46	0.08	0.19
	A 2008	-	-	0.78 $^{+}$*	0.48	0.19	0.51	0.71	0.66	0.31	0.70 $^{+}$
	A 2009	-	-	-	0.50	0.29	0.53	0.42	0.90 $^{+}$*	0.29	0.48
	Sp 2008	-	-	-	-	0.60	0.14	0.32	0.37	0.71 $^{+}$	0.63
	Sp 2009	-	-	-	-	-	0.13	0.18	0.19	0.72 $^{+}$*	0.70 $^{+}$*
HE	**A 2007**	-	-	-	-	-	-	0.66	0.54	0.24	0.08
	A 2008	-	-	-	-	-	-	-	0.37	0.43	0.56
	A 2009	-	-	-	-	-	-	-	-	0.15	0.33
	Sp 2008	-	-	-	-	-	-	-	-	-	0.56
	Sp 2009	-	-	-	-	-	-	-	-	-	-

B

		LE					HE				
		A 2007	**A 2008**	**A 2009**	**Sp 2008**	**Sp 2009**	**A 2007**	**A 2008**	**A 2009**	**Sp 2008**	**Sp 2009**
LE	**A 2007**	-	*0.21*	*0.23*	*0.24*	*0.92*	*0.0001*$^{+}$*	*0.009*	*0.06*	*0.78*	*0.49*
	A 2008	-	-	*0.0001*$^{+}$*	*0.04*	*0.47*	*0.24*	*0.002*	*0.004*	*0.21*	*0.001* $^{+}$
	A 2009	-	-	-	*0.03*	*0.23*	*0.25*	*0.10*	*0.0001*$^{+}$*	*0.24*	*0.04*
	Sp 2008	-	-	-	-	*0.008*	*0.60*	*0.22*	*0.13*	*0.001* $^{+}$	*0.005*
	Sp 2009	-	-	-	-	-	*0.45*	*0.51*	*0.44*	*<0.001*$^{+}$*	*<0.001*$^{+}$*
HE	**A 2007**	-	-	-	-	-	-	*0.01*	*0.03*	*0.24*	*0.76*
	A 2008	-	-	-	-	-	-	-	*0.16*	*0.10*	*0.02*
	A 2009	-	-	-	-	-	-	-	-	*0.55*	*0.16*
	Sp 2008	-	-	-	-	-	-	-	-	-	*0.02*
	Sp 2009	-	-	-	-	-	-	-	-	-	-

Table 5-1-3 **Correlation in relative performance of families between all growth experiments at LE and HE from 2007 to 2009**: (**A**) Correlation coefficients (r), (**B**) *p*-values for the relative performance not being correlated, without Bonferroni correction. Correlations that remain significant at p = 0.05 and p = 0.01 after Bonferroni correction are shown by $^{+}$ and *, respectively. Keys: **A**, autumn; **Sp**, spring; **LE**, low elevation; **HE**, high elevation.

GxE interactions among genotypes, elevations and seasons

Table 5-1-4 summarizes the analysis of GxE interactions for all plants grown at LE and HE in autumns and springs. Normalized growth data from all experiments were used for the analysis - normalization should eliminate variation between elevations and seasons for each experiment. However, normalization should not obscure any interactions between genotype and environmental factors; for example, genotypes can differ in their relative growth performance between experiments, even though the mean of growth rate and variance for each experiment has been made the same through normalization.

Variance across groups (MS) was highly significant for genotype and season ($p < 2.2E-16$ and $p = 1.96E-13$, respectively), but not elevation. This suggests genotypes and season explain a significant proportion of variance in growth rate (Table 5-1-4). There are also interactions between genotype and season, as well as elevation (though it explains less variance than season), and a smaller interaction encompassing all three ($p < 2.2E-16$, = 1.96E-13 and 5.12E-5, respectively). This supports the concept of local genotypes being seasonally adapted.

	df	SS	MS	F	p
Genotype (or family)	73	1034.46	14.1707	31.7183	< 2.2E-16
Season (autumn and spring)	1	24.44	24.4386	54.7007	1.96E-13
Elevation (LE and HE)	1	0.01	0.0082	0.0185	0.8919
Genotype : Season	39	290.27	7.4429	16.6593	< 2.2E-16
Genotype : Elevation	70	96.69	1.3813	3.0917	6.90E-16
Season: Elevation	1	0.08	0.0777	0.1740	0.6766
Genotype : Season : Elevation	37	36.15	0.9771	2.1870	5.12E-05
Residuals	2294	1024.89	0.4468		

Table 5-1-4 **GxE for seasonal growth at LE and HE**. GxE interactions are present among genotype, season and elevation. Key: **LE**, low elevation; **HE**, high elevation, **df**, degree of freedom; **SS**, sum of square; **MS**, mean square.

Individual seasonal growth summary

The growth of 20 accessions in the different seasonal experiments was ranked and summarized in Figure 5-1-4. Some families were relatively fast growing in both autumn and spring, whereas some grew relatively slowly in both. Families 5A3, 10A1 and 10B1 were among the fastest growing families, regardless of season or elevation, while families such as 2E1 and 12A1 were ranked as the slowest growing in most seasons and sites. Although 5A3 was one of the fastest growing families at LE and HE regardless of season, a slightly different growth performance was noted in the greenhouse – it ranked lower in autumn than in spring.

Strikingly, the ranking results also indicate that some families performed consistently better in autumn than in spring (e.g., 11B2, 13B6), and others better in spring than autumn (e.g., 4A4 and 8A4; Fig. 5-1-4). In addition, more closely related families were more likely to grow at similar rates. For example, families 11A2 and 11A5 both grew moderately fast, whereas 2C2 and 2E5 were both moderately slow growing.

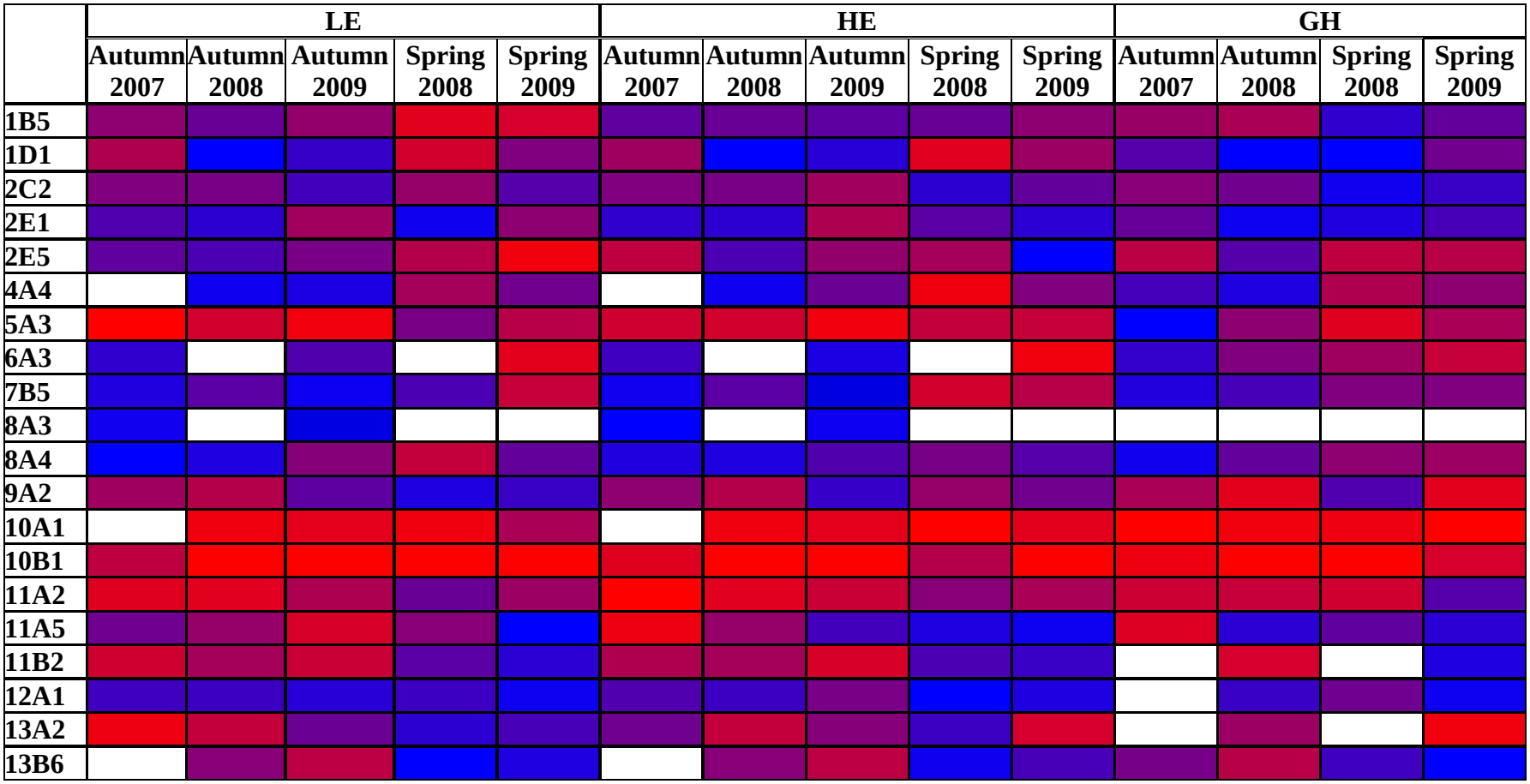

Figure 5-1-4 **Seasonal growth rank**. 20 families were coloured to summarize their relative ranking of growth rate based on average rosette areas. Red represents the fastest growth and blue represents the slowest. Some families, 10A1 and 10B1, were the fastest growing families in the population in all seasons and elevations, whereas 12A1 and 2E1 were the opposite.

Discussion

The results of this chapter illustrate the potential importance of elevation and seasonal climate on the growth of local *Arabidopsis thaliana* accessions.

The significance of high heritability, H^2, in rosette growth

Growth rate variation appeared to have an underlying genetic basis, as evidenced by its high heritability. High H^2 estimates in Table 5-1-1 A suggest that most of the variation was genetically determined in each experiment and there was no obvious difference in the estimates of H^2 between spring and autumn. Strictly speaking, these experiments estimate the repeatability, R, of growth rate. R accounts for the effects of environmental variance: $R = (V_G+V_{Eg}) / V_P$, where V_{Eg} is general environmental variance, so R corresponds to H^2 when V_{Eg} tends to zero and R provides an estimate of the upper limit of H^2 (Falconer & Mackay 1996). In performing the experiments, multiple replicates were used per family (genotype) and, as far as possible, plants were grown in the same environment within an experiment and attempts were made to avoid confounding genotype and any residual environmental variation by randomizing the arrangement of plants. Therefore V_{Eg} is assumed to be small so R values approximate to H^2.

Heritability indicates the level of phenotypic variation that is attributable to genetic variance. In order for evolutionary adaptation to occur, a population must have phenotypic variation due to genetic variance, such that natural selection acts upon those phenotypes conferring a reproductive advantage. Thus, heritability is a vital component in the process of adaptation.

Local adaptation occurs when selective pressure varies across the landscape, for example for factors such as elevations (Knight 2004), temperature, water availability (Dudley 1996), competition and herbivory (Sork, Stowe and Hochwender 1993). The growth

differences observed in this study may represent adaptations to different environments from which the local accessions were sampled.

Growth performance in different environments

I. Different elevations

The environmental differences between the sites had an effect on growth rate. GH plants grew faster than LE and HE field plants in both seasons. This is consistent with more hospitable conditions at GH, where temperature never reached freezing (the lowest temperatures were approximately 4°C and 6°C in the winter and spring, respectively); temperatures were generally warmer and plants were protected from wind and herbivores and had an ample water supply.

In general, LE plants usually grew faster than their HE cohorts; the 2°C higher average temperature at lower elevations may have some influence in this. However, frost and ice formed earlier at HE than LE in autumn 2008 and snowfalls were heavier and snow cover lasted longer at HE than LE during the winter of 2009. Sub-zero temperatures combined with the corresponding lack of free liquid water may restrict growth. However growth at LE and HE largely reflected weather conditions so growth rate in the same season could very from year to year when the weather differed between years. There were some exceptions to the trend for faster growth at LE, possibly due to other factors such as herbivory. For example, plants at LE suffered from herbivory by slugs in 2007, possibly clearance of the surrounding vegetation had removed alternate food sources.

Although the differences in environment between the LE and HE sites were sufficient to affect plant growth, they did not significantly affect the relative performance of families. Families were sampled from a range of elevations and if they were adapted to conditions that varied with elevation those from higher elevations would be expected to perform better that those from low elevations when grown in common garden conditions at HE,

and vice versa. However, the strong correlations between growth rates at LE and HE within the same year (i.e. $r = 0.71$, $p = 0.001$), suggest that this is not the case.

II. Different seasons

Seasonal variation had a significant effect on plant growth (Table 5-1-2). In GH, plants grew marginally slower in the autumn than in spring cohorts. This could be a consequence of the lengthening photoperiod in spring, since other variables, such as water availability, wind speed and temperature in GH were similar between spring and autumn. However, at both field sites plants grew consistently faster in the spring than in the autumn. This might reflect differences in various environmental factors (e.g., ambient temperature, light intensity, day length and water availability). Temperature was potentially the most important factor: for example, growth was slower in the colder autumn of 2008 than in the warmer autumn of 2009. A similar effect was seen when comparing growth rates between spring 2008 and the colder spring of 2009.

Although seasonal growth performance is less predictable in the field, LE and HE growth strongly correlated within either autumn or spring: the majority of families showed similar performance at both sites during the same season. There was no significant difference between the strength of these growth rate correlations in autumn and those in spring.

Adaptation to the environmental variables associated with a particular season is suggested by the lack of correlation between the growth rate of accessions autumn and in spring. The lack of correlation cannot be explained by a reduced contribution of genetic differences to growth rate variation in one season, because heritability estimates are similar in spring and autumn. Some families performed relatively well in one season and poorly in the other. This implies that different families might be adapted to seasonal differences in climate.

One exception to the lack of correlation between performance in autumn and spring was observed at LE for autumn 2008 and spring 2009 ($r = 0.70$, $p = 0.001$). These two periods were unusual for different seasons in sharing similar temperatures (Chapter 4), further supporting the idea that local accessions might be adapted to different temperatures.

Adaptation to seasonal variables

The relative performance of different *A. thaliana* accessions could vary consistently with seasonal cues. This discovery has not been reported previously: usually, *A. thaliana* has simply been described as having slow growth over a long period when germinating in autumn and fast growth as annuals when germinating in spring or summer. All genotypes respond to the different environments experienced in different seasons, but the response differs between genotypes.

A pattern of climatic adaptation has been reported for *A. thaliana* from a common garden experiment carried out by Rutter and Fenster (2007). Standard soil was used in the experiment, other vegetation was removed by covering it with a landscape cloth and plants were watered. They detected heritable differences in fitness between accessions and found that accessions had higher relative fitness when they had originated from a similar climate and latitude to their experimental ground. This strongly suggests that *A. thaliana* can adapt to local environmental factors such as day length, light quality or intensity.

The results presented in this section are consistent with adaptation to factors that vary between seasons, as supported by significant genotype x season interaction (Table 5-1-4). However it is not clear which of the environmental factors that vary between seasons are involved.

5-2 Seasonal flowering time

The transition from vegetative growth to the reproductive phase is a major event in an annual plant's life cycle – the timing is crucial for successful seed production. *A. thaliana* is usually described as a long day species, where most world wide ecotypes flower earlier under long day than under short day conditions. Examining flowering time of local accessions under various combinations of season and elevation, offers the potential to better understand the influence of natural conditions upon local populations' reproductive success.

Effects of elevation and season on flowering time

During the experimental period (autumn and spring), whilst growth was being recorded, the vegetative shoot apex of all plants (particularly those early flowering families) that survived were visually inspected. These inspections took place at initially at weekly intervals, before shortening to every 2 to 3 days either after first bolting was observed or upon the onset of warmer weather. Bolting time was recorded as soon as an inflorescence bud (approximately 3 mm diameter, with the bud shaped like a small pearl) was visible. The flowering time was recorded as being the point where the first flower opened.

Figure 5-2-1 shows a significant linear correlation ($r = 0.99$, $p < 0.0001$) between bolting and flowering times under controlled conditions (20°C LD, high light): all accessions flowered within approximately four of days of bolting, whether they were early or late flowering genotypes. This indicates that flowering time is directly related to bolting under controlled conditions. For example, members of early flowering family 9A2 bolted around 21 dsg and flowered within three to four days of bolting. Although the most late flowering family, 10A1, bolted over a month later than 9A2 (at around 54 dsg) it too flowered around three to five days after bolting.

Compared to plants in growth chambers, autumn field-grown plants showed different relationships of bolting to flowering. Figure 5-2-2A shows that plants in autumn 2008 began to bolt at between 70 and 160 dsg but flowered from 160 dsg onwards. They bolted in three different groups: early, intermediate and late. The early flowering accessions bolted between 74 and 81 dsg, whilst the intermediate accessions bolted between 100 and 123 dsg. Finally, the late flowering accessions bolted between 151 and 158 dsg. Despite the distinct differences in bolting times, all families flowered between 160 and 190 dsg (mid March to April). Therefore plants that had bolted earlier in the experiment, during early winter (mid December), took longer to progress from bolting to flowering. A similar effect was seen at HE, with plants bolting over a period of approximately 12 weeks but flowering within a three week window, from end of March until mid April (data not shown).

In the autumn of 2009, which had warmer temperatures than 2008, a different pattern of bolting and flowering was observed (Fig. 5-2-2B). Plants at LE began to bolt from 44 dsg (the end of October) to 186 dsg (mid March). Plants that bolted in mid autumn were able to progress to flowering in late autumn; early flowering family 9A2 bolted around 44 dsg and took an average of 19 days to flower. Those that bolted from late autumn to early winter did not flower until the following spring, in mid March. The latest bolting families both bolted and flowered within a short time window in spring (mid March to end of April). A similar pattern of bolting and flowering was also observed field populations in autumn 2007 (data not shown).

The autumn bolting and flowering pattern observed in the field suggests that the flowering time variation detected under controlled conditions has little importance in cold autumns, because flowering times were not directly related to bolting times. However, it might be significant in mild autumn, since early bolting plants were able to flower early in the mild autumn of 2009.

Plants in spring seasons, however, have a bolting and flowering pattern that is more similar to plants grown under controlled conditions. A highly significant linear

correlation was observed at GH in spring 2009, $r = 0.89$ ($p < 0.0001$). Plants at GH bolted between 36 dsg and 70 dsg, starting in late April. Similar to plants under controlled conditions, early families bolted just over a month before late flowering families. In spite of the bolting onset in GH being delayed by two weeks compared to plants in a controlled chamber, early and late flowering families flowered in a relatively fixed interval period within two weeks after bolting.

Both spring 2008 and 2009 LE populations showed a similar pattern of bolting and flowering to the GH population. These plants bolted slightly later than those in GH, at between 42 dsg to 72 dsg, and flowered three to six weeks later. Plants at HE bolted and flowered approximately five to seven days later than at LE (data not shown).

Overall, the intervals between bolting and flowering time were more predictable in spring than autumn and were more similar to those of plants grown under controlled conditions. This implies that genetically controlled differences in the reproductive transition had little effect on flowering time in colder autumn conditions, but a greater influence in the warmer spring climate.

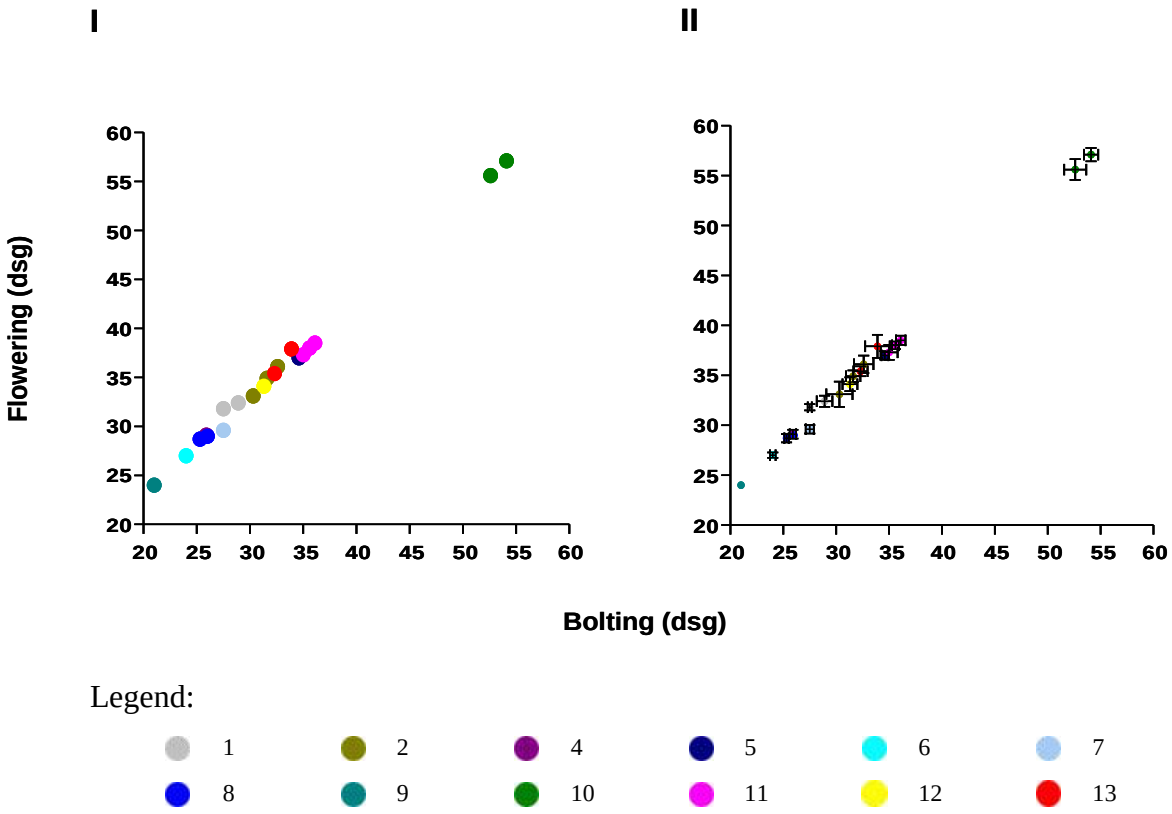

Figure 5-2-1 **Linear regression of bolting against flowering under controlled conditions**. Plants grown under controlled condition (20°C LD, high light) flowered three to five days after bolting, resulting a significant correlation between the two developmental transitions, $r = 0.99$ ($p < 0.0001$). Mean values of nine family members are shown with families colour coded based upon the sampling locations 1 to 13 (**I**) ± *SE* (**II**).

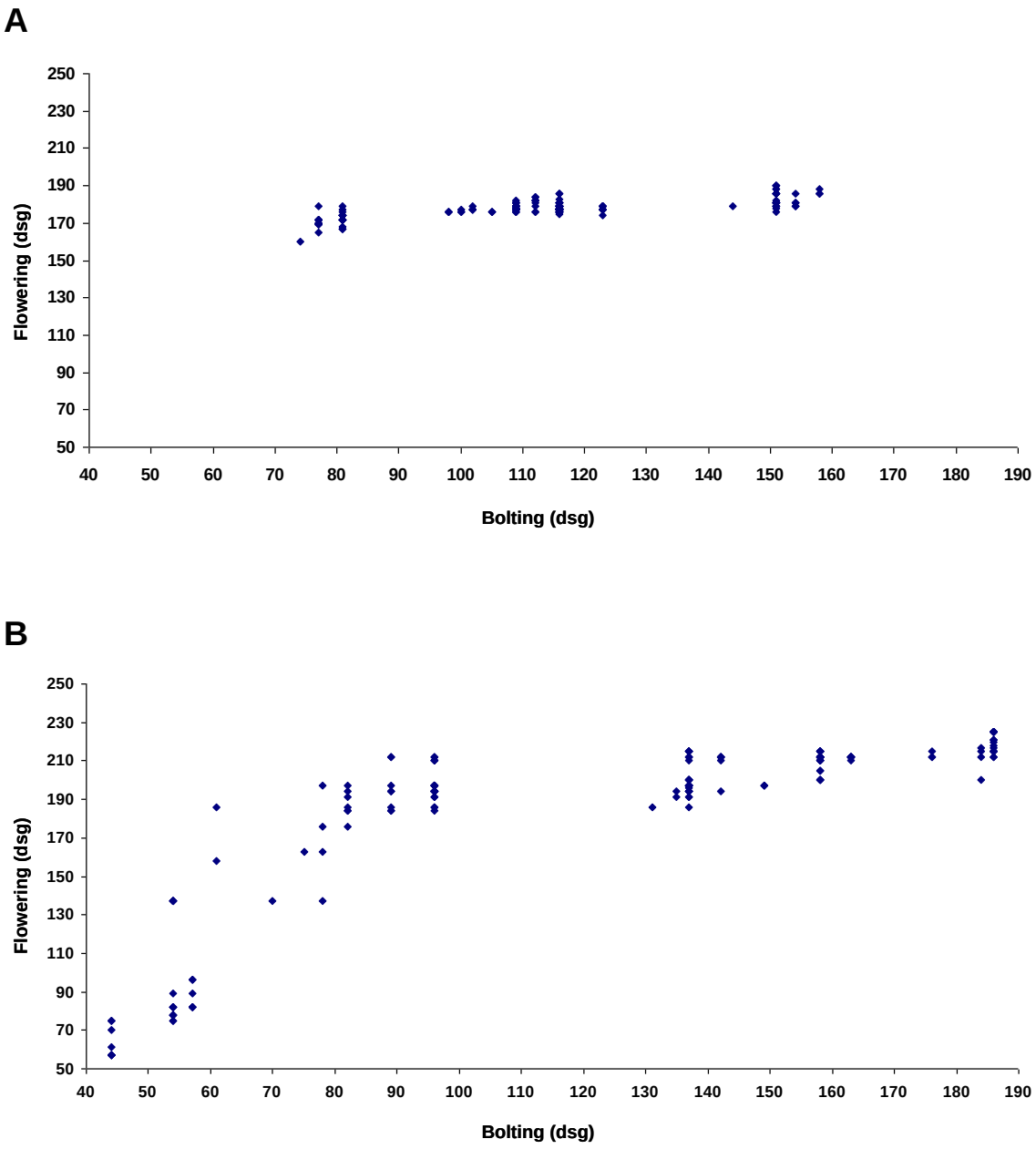

Figure 5-2-2 **Regression of bolting against flowering under natural conditions at LE in autumn 2008 (A) and 2009 (B)**. Plants in the field did not show a fixed interval between bolting and flowering. Bolting and flowering in autumn 2009 exhibits a significant non-linear correlation. Population sizes in autumn 2008 and 2009 were 191 and 175 plants, respectively. **(A)**: $r = 0.75$, $p < 0.0001$ and **(B)**: $r = 0.80$, $p < 0.0001$.

The relationship between growth and reproduction

The relationships between rosette growth (area) and bolting/ flowering time were estimated. A weak but marginally significant positive correlation was found between growth rate and bolting time in plants grown under controlled conditions (20°C LD high light); $r = 0.48$, $p = 0.03$; Fig. 5-2-3). A similar correlation was found between growth rate and flowering time (graph not shown). This suggests that fast growing accessions do not necessarily flower earlier. Conversely, slow growing plants do not always flower later.

In order to account for the possible biasing effects of extreme (outlier) families upon the positive correlation, further comparisons were made. If the fast growing, late flowering families (10A1 and 10B1) were excluded, an equally strong correlation was determined ($r = 0.52$, $p = 0.03$). Similarly, if both extreme late and early flowering families were excluded, the relationship between bolting and growth is stronger and more significant: $r = 0.67$, $p = 0.003$. This shows that the strong correlation is not dependent on the extreme outliers.

Compared to plants grown under controlled conditions, the relationship between growth and bolting time of autumn field-grown plants was more significant. As shown in Figure 5-2-4A and B, rosette growth and bolting correlated significantly at LE in autumn 2008 and 2009, where $r = 0.53$ and 0.54, respectively ($p < 0.0001$). GH plants (autumn 2008) were also determined to have show a significant correlation between growth and bolting ($r = 0.42$, $p < 0.001$). Interestingly, significant but weaker relationships between growth and flowering time were found at LE in autumn 2008 and 2009; $r = 0.16$, $p = 0.02$ and $r = 0.27$ and $p = 0.0003$, respectively. This indicates that autumn rosette growth rate is a better predictor of bolting time than of flowering time.

As with the results from controlled conditions, two sets of extreme outliers were defined: firstly, the fast growing and late flowering families and the second outlier set was composed of the earliest and latest flowering families (flowering age).

Autumn 2008 indicated outlier families at LE did have some effect upon the correlation estimations. The relationship between growth and bolting remained significant ($p < 0.0001$ in both cases), but weakened with the exclusion of the outlier sets ($r = 0.35$ and 0.34 for fast growing/late flowering and extreme flowering age sets respectively). A more pronounced effect was observed upon the relationship between growth and flowering time; removal of both fast growth/late flowering and extreme flowering age set resulted in a notable lack of correlations, $r = 0.06$ ($p = 0.45$) and 0.07 ($p = 0.36$), respectively.

In autumn 2009 period, the correlation between growth and bolting at LE remained significantly positive for removal of both sets of outliers (in both cases $r = 0.43$, $p < 0.0001$). Similarly, the relationship between growth and flowering remained the same and significant after the removal of both outlier groups ($r = 0.22$, $p < 0.02$). This indicates these outliers were not biasing the overall correlation results for this time period.

It is possible that the colder recorded environmental conditions in autumn 2008 (as compared to 2009) may have had an influence upon flowering time beyond that of growth rate. Nonetheless, these results further assert rosette growth rate is a better predictor of bolting time than flowering time in autumn.

Growth and bolting time in the field exhibited a different relationship in spring compared to autumn. Growth and bolting time at LE in spring 2009 were uncorrelated ($r = 0.08$, $p = 0.35$). After removing the fastest growing and late flowering families, a weakly negative but highly significant correlation of growth and bolting was observed ($r = -0.38$ $p < 0.0001$). This negative correlation was further strengthened when both extreme early and late flowering families were eliminated ($r = -0.49$, $p < 0.0001$). Without these exceptional performers, the results suggest that faster growing individuals reach a reproductive stage earlier than slower growing individuals in spring.

A marginally positive correlation was observed at GH in the same time period ($r = 0.21$, $p = 0.05$). However, a similar weakly negative but highly significant correlation of growth and bolting (to LE spring 2009 set) was observed ($r = -0.40$ $p = 0.0002$), after removing the fastest growing and late flowering families from the experimental population. The elimination of both extreme early and late flowering families did not further alter the relationship between growth and bolting ($r = -0.42$, $p < 0.0001$).

In spring 2008, a stronger and more significant positive relationship was observed at GH ($r = 0.42$, $p = 0.002$) compared to spring 2009. The removal of the two sets of outliers broke the relationship between growth and bolting - growth and bolting became uncorrelated, $r = 0.08$ and 0.004 ($p = 0.61$ and 0.98) respectively. This further indicated the importance of climate required to maintain the relationship between growth and bolting in different seasons.

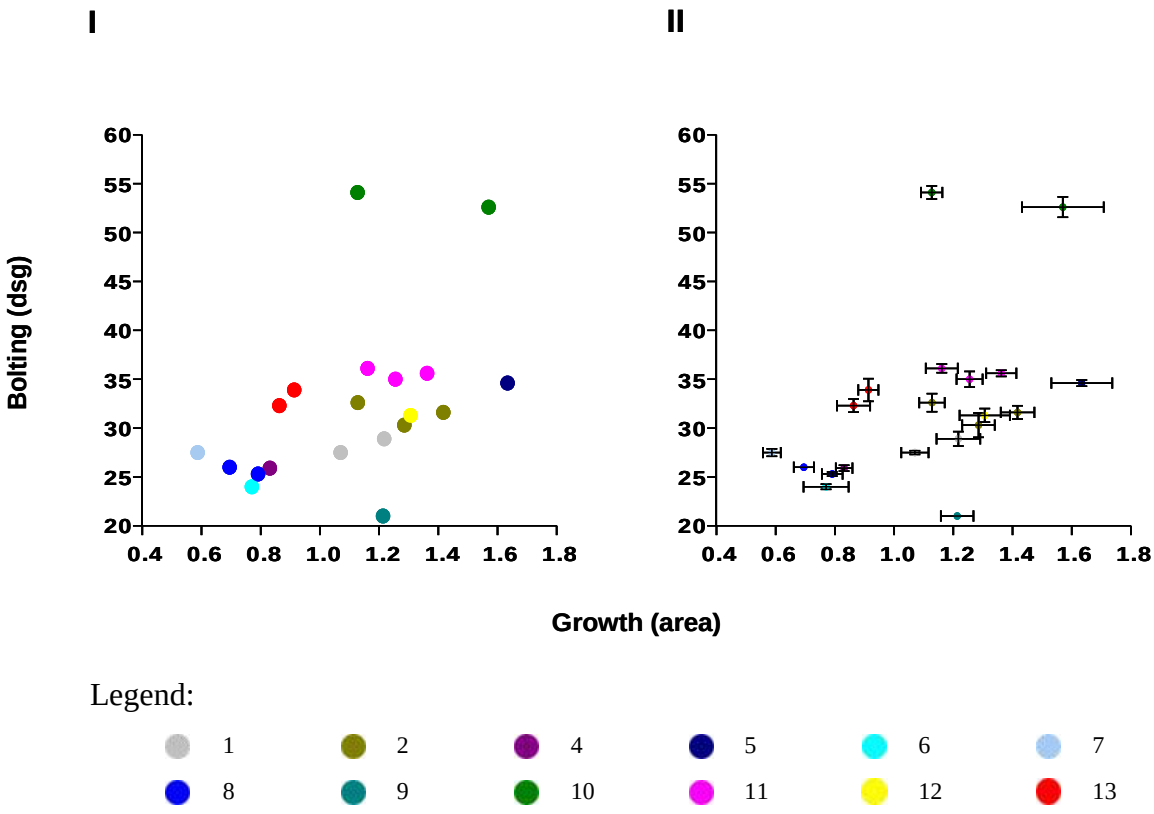

Figure 5-2-3 **Regression of growth (rosette area, cm^2) against bolting time (dsg) under controlled conditions**. Growth and bolting time of chamber plants exhibit a weak but marginally significant correlation, r = 0.48 (p = 0.03). Mean values are shown with families colour coded based upon the sampling locations 1 to 13 (**I**) ± *SE* (**II**).

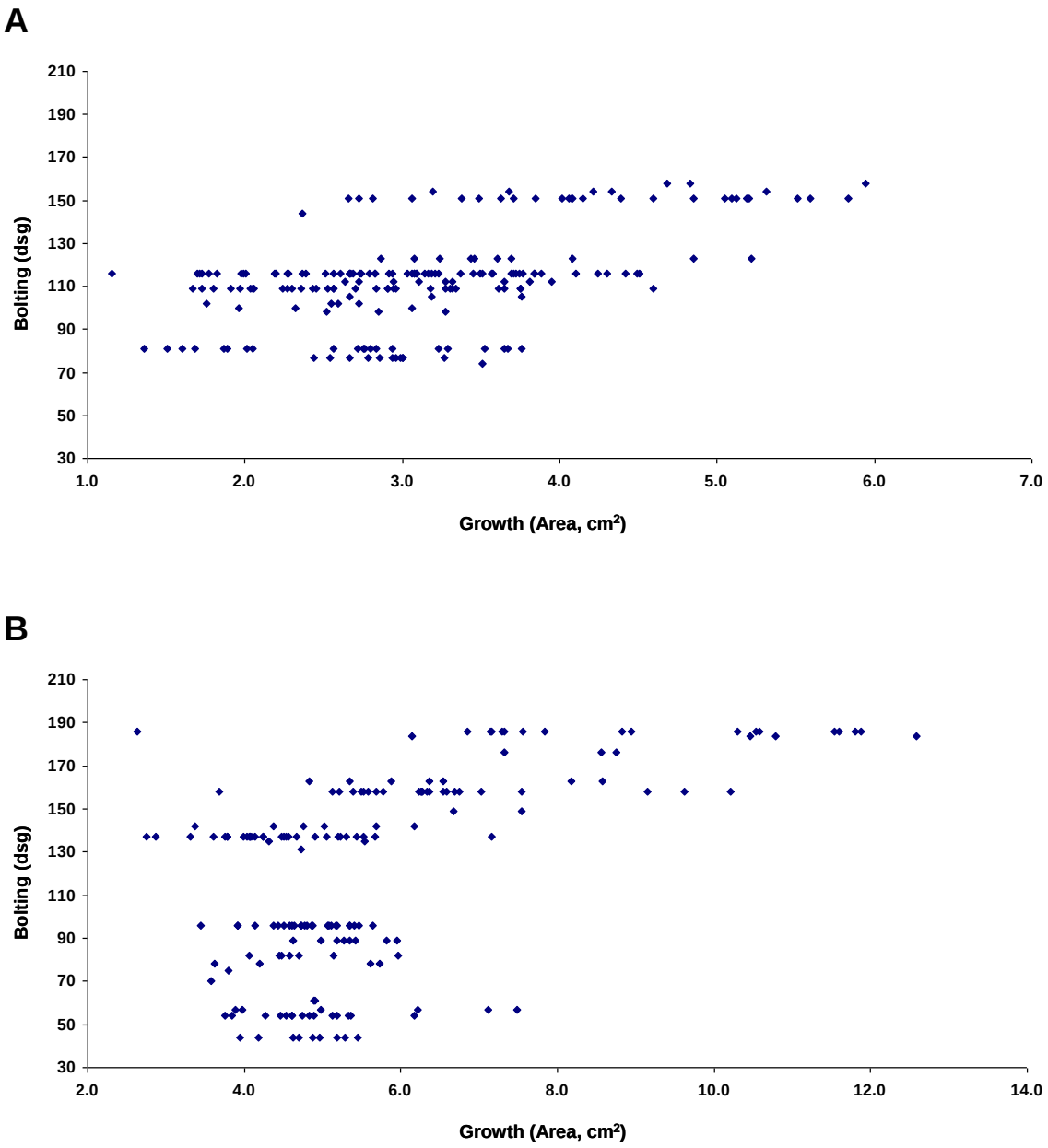

Figure 5-2-4 **Regression of growth (rosette area, cm^2) against bolting time (dsg) under natural conditions at LE in autumn 2008 (A) and 2009 (B)**. A weak but significant correlation between growth and bolting time was observed in LE populations in different autumns. Both populations gave similar r-values (0.53 and 0.54) and $p < 0.0001$. Population size in autumn 2008 and 2009 were 191 and 175 respectively.

Discussion

The significance of bolting and flowering relationships in different conditions

Bolting and flowering (opening of the first flower) were found to be strongly and directly related when measured under controlled conditions (20°C LD, high light). Bolting (determined as the point where the inflorescence bud is first visible), in this case, can be used as an indicator of the beginning of the reproductive stage.

Under favourable conditions, it is not surprising to see a smooth change within a developmental stage. The significant correlation between these two developmental phases, regardless of whether the genotype is early or late flowering, is intriguing. This suggests that the variation in the onset of bolting (or flowering) is likely to be genetically determined, but the regulation of bolting to flowering transition is affected in a similar manner for all local genotypes under optimum growth conditions. A recent report on bolting and flowering phase change by Pouteau and Albertin (2009) suggested bolting to flowering transition under LD conditions may be coordinated by the FLOWERING LOCUS T (FT) pathway. FT is known to induce flowering by acting as a mobile flowering signal, and under LD, FT expression is up-regulated (King *et al*. 2008; Zeevaart 2008).

Autumn (field-grown) plants would initiate the transition to reproductive stage under short day length - less than 12 hours of photoperiod if the temperature was suitably warm (observed as between 6°C and 14°C). This behaviour is significant in milder autumns, such as in 2007 and 2009, where the seasonal average minimum and maximum temperatures were in a suitably warm range. Although plants bolted later than they would under LD chamber conditions, early flowering families in autumn 2009 did enter their reproductive stage – they bolted under 10 hours (late Oct) of day length and the first flower opened under approximately 8.5 hours of day length (Nov, late autumn).

This behaviour suggests that bolting is not dependent on photoperiod and can be easily overwritten by other flowering pathways under natural seasonal conditions, at least in these early flowering genotypes. Bolting and flowering observed in the SD autumn plants could be induced by the sugar and gibberellins signaling pathway, as FT does not usually operate under SD (Pouteau and Albertin 2009; King *et al*. 2008; Eriksson *et al*. 2006; Blazquez 2003).

However, those intermediate flowering families that bolted around the 8.5 hours of day length did not flower until the day length returned to (approx.) 12 hours. This suggests that bolting is less sensitive to temperature than the progression from bolting to flowering.

Wilczek *et al*. (2009) have created a photothermal model, predicting transition from rosette to bolting under controlled conditions that explain the flowering variation observed in field experiments. They have shown that once the required threshold of environmental determined photothermal developmental units was met, plants can proceed into transition from vegetative to reproductive growth (bolting). This suggests another explanation for this phenotypic variation: these local accessions maybe vary in sensitivity towards natural environmental variables, which could act in conjunction with known flowering signaling pathways.

This may possibly better explain the significant bolting/ flowering observation in autumn field experiments, as it is well known that flowering time is regulated by a network of genes whilst integrating and responding to multiple environmental signals (reviewed by Koornneef 2004). If early flowering families are more sensitive towards temperature (for example) that could allow more efficient accumulation of energy for bolting and flowering. Conversely, other less temperature sensitive individuals may require a longer time period to achieve the energy threshold, and thus only be able to bolt before the onset of harsher seasonal conditions.

During colder autumn conditions (i.e. 2008), environmental resources may be too scarce for any genotype to accumulate sufficient photothermal units. In this case, it may be that even the best genotype is one that is able to best conserve energy, in order to wait until change in environmental conditions. Nonetheless, it was unclear whether early flowering families actually set viable seeds in the period before winter, as they would in spring.

Genetically controlled differences in the reproductive transition have a clearer influence on plants grown in spring than in autumn. Bolting and flowering time observed in spring was predictable and similar to those plants grown under warm LD control conditions. Day length at the time bolting was observed as approximately 15 hours, similar to the photoperiod of the LD control.

If FLOWERING LOCUS T was the main flowering inducer under controlled LD conditions, it can be considered as a candidate responsible for bolting and flowering in spring plant. It is unlikely that vernalization plays a role in inducing flowering in spring populations, although it is well documented to induce flowering. Experimentally, vernalization is induced with a prolonged (1-3 month) period at around 4°C and therefore might not have occurred during the spring experiment: when the seedlings were was introduced to the field, the average daily temperature was above 4°C and warmed up steadily from then onwards (Chapter 4). However, it is possible that temperatures higher than 4°C are able to advance flowering by vernalization.

Different bolting and flowering patterns observed from the seasonal experiments strongly suggest that environmentally dependent expression of allelic variation may have important implications for life history in natural annual populations - such as in these local *A. thaliana*.

The significance of growth and reproduction

The relationship with rosette growth and bolting/ flowering time was significant under warm LD controlled conditions. In spite of the existence of extreme outliers in the population, such as (for example) early flowering genotypes, it is possible to predict transition to reproduction based on the growth rate under optimum controlled conditions.

Under natural conditions, relationships between rosette growth and bolting time were often significant. However, the strength varied occasionally with seasonal conditions: differences were observed in results for as autumn and spring.

The strength of growth and bolting relationships at LE during autumn (both 2008 and 2009) was observed to be dependent on families in the population with extreme phenotypes. The relationship between autumn growth and bolting was strengthened by extreme families in 2009 (from r = 0.43 to 0.54 when extreme families were included) and even more dramatically in colder autumn 2008 (from r = 0.35 to 0.53).

One possibility is that both growth rate and bolting in those extreme families may be affected by similar genes. The results of correlations also suggest that these gene actions may be temperature dependent; lower temperature may have an effect in strengthening the influence of these genetic factors upon growth and bolting time (as observed in 2008).

Méndez-Vigo *et al* (2010) reported a number of quantitative trait loci (QTL) that affected flowering initiation as well as the rate of leaf production using Landsberg *erecta* × Fei-0 recombinant inbred lines (RILs). They found that all - except one - genomic regions affecting flowering time, also affected leaf production (rosette growth) in some periods of vegetative growth. This further supports the suggestion that high positive correlation, stemming from the inclusion of the extreme families in autumn 2008, is the result of a similar genetic influence: these extreme families may possess (or a combination of) genes that cause both high growth rate and late flowering time.

Alternatively, different genes might regulate growth and bolting independently, in which case the correlation could result from the presence of fast growth and late flowering alleles (and vice versa) in the same individuals. This might be chance or might be the result of selection for fast growth and late flowering.

In spring, the inclusion of extreme families disrupted the correlation between rosette growth and bolting. Removing those extreme families led to highly significant negative correlation: faster growing individuals reached reproductive transition earlier than slower individuals. This suggests that genetically controlled differences in reproductive transition had great influence in the warmer spring climate.

This different characteristic response may be explained through the environmental changes in spring compared to autumn and winter. The change of seasons offers both a reduction in stressors such as (low) temperature and an increase in available resources with the onset of longer daylight hours and greater availability of liquid water. It may entail that faster growth, combined with early transition to flowering, is advantageous in spring as a step to avoid drought periods in the warm summer months.

5-3 Seasonal seed yield

Fitness can be defined as the proportion of the next generation that descends from an organism - i.e. the number of its offspring as a proportion of all offspring in the subsequent generation. There is a potential difference between physiological performance and evolutionary fitness; the latter only becomes apparent with time, which represents a long wait for organisms with a long generation time.

It is difficult to measure the fitness of outbreeding plants: whilst the mother is obvious (as the seed bearer), the father may be any one of a number of pollen donors (it is obviously very difficult to track the movements of pollen or pollinators or to identify the likely father of a plant's seeds). Highly selfing species, such as *A. thaliana*, are more suited for fitness measurement as the seed bearing plant is also likely to be its own pollen donor: meaning that fitness comparison does not involve identifying the father.

The assumption has been made here that the genetic contribution of one individual to the next generation is directly related to the production of seeds by that individual. That is, that an individual producing a greater proportion of seeds in one generation will contribute a greater proportion of the offspring in the next.

To estimate the fitness of individual accessions from seasonal experiments, seeds were collected at maturity and then weighed. The number of seeds produced by each individual in the experiment could then be used as an estimate of relative fitness.

Seed yield from different sites and seasons

Seed yield was observed to vary in different environmental conditions. Table 5-3-1 shows the average seed yield per plant for all individuals that survived to fruit at GH, LE and HE, as measured during seasons over the experimental period. The composition of these populations was not exactly the same because a few families were not included in

some experiments either because they failed to germinate or because they failed to flower. However, most families were successfully grown and flowered in all experiments and the experiments are therefore broadly comparable.

In general, populations that grew under more hospitable conditions (those sheltered at the GH location) produced more seeds than those grown in the field. This is supported by the observation that plants in GH produced a significantly greater yield compared to plants at LE and HE ($p < 0.0001$) in both autumn and spring (Table 5-3-1). For example, GH plants could yield as much as 18 times the seeds of plants from the field populations in autumn (i.e. 0.512 g at GH versus 0.027 g at HE in autumn 2008). The yield differences between GH and field populations were less in spring compared to autumn - spring GH seed production was approximately 2 to 4 times more than from LE or HE. Unsurprisingly, the seed yield difference between elevations (LE versus HE) was not as dramatic as seen between indoor and outdoor conditions. The average seed yield per plant was higher at LE compared to HE in five out of the six experiments (Table 5-3-1).

When comparing seed yields between seasons, the average was greater in spring sown populations than in autumn populations. This was true regardless of whether the plants were sited at LE, HE or GH (Table 5-3-1). For example, spring sown plants at GH produced 1.2 to 1.7 times greater seed yield ($p = 0.06$ and < 0.0001 respectively) than the autumn cohorts in 2008 and 2009.

A greater yield difference between seasons can be observed for field populations. Plants grown in the field produced significantly more seeds in spring than autumn: at least twice as many in the spring 2008 population ($p < 0.0001$) than the autumn 2007 population, and approximately 10 times more in spring 2009 than autumn 2008 ($p < 0.0001$). In addition, plants grown in the warmer autumn and spring of 2009 also produced more seed than those in colder autumns and springs of 2007 and 2008 (Chapter 4; Table 5-3-1).

	2007		2008				2009			
	Autumn		Spring		Autumn		Spring		Autumn	
	Seed yield (g)	***SE***	***Seed yield (g)***	***SE***	***Seed yield (g)***	***SE***	***Seed yield (g)***	***SE***	***Seed yield (g)***	***SE***
GH	0.462	0.001	0.537	0.035	0.512	0.048	0.856	0.048	-	-
LE	0.032	0.001	0.118	0.004	0.035	0.001	0.365	0.016	0.182	0.004
HE	0.060	0.002	0.106	0.003	0.027	0.002	0.263	0.008	0.076	0.003

Table 5-3-1 **Average seed yield (g) of individuals in different seasons and sites**. Values are the mean seed yield from plants that survived to fruiting, with their standard errors (SE). Plants generally produced more seeds from spring sowing than from autumn. In addition, GH plants produced more seeds than LE and HE combined in either autumn or spring.

Seed yield from selected families at different sites and seasons

In order to test for any association between seed yield and growth rate in different seasons, 20 families were selected for further examination based on their range of growth rates, from fast to slow. An absolute value for seed yield was recorded for each surviving individual in the experiments. The average seed yield per family from all experiment sites between autumn 2007 and 2009 was calculated and is summarized in Table 5-3-2.

In summary, most plants from selected families that were grown in GH (except families from site 10) produced more seeds in spring than in autumn (Table 5-3-2 GH). However, the highest and lowest average family seed yields of any season were also recorded in spring. For example, the greatest yield from GH grown plants in all autumns was approximately 0.75 g (2E1, in autumn 2008) whereas the lowest was 0.29 g (12A1, in autumn 2008). In spring, the greatest seed yield was approximately 1.14 g (2E5, in spring 2009) and the lowest, 0.19 g (12A1, spring 2008).

Seed production from field grown plants at LE and HE was more variable than in their GH cohorts (Table 5-3-2 GH, LE & HE). Although the general seed production exhibited a similar pattern to that seen at GH - most plants produced more seeds in spring

than in autumn - plants were seen to produce almost as many seeds in autumn as in spring on some occasions. For example, the range of average family seed production at LE in spring 2008 is 0.033 g (10A1) to 0.245 g (2E5), whereas it was 0.109 g (8A3) to 0.276 g (10B1) in autumn 2009 (Table 5-3-2 LE). It could be suggested that this pattern reflects climate conditions that were more similar between these two seasons than is normal.

Similar to GH, field plants were observed to have the greatest range between highest and lowest seed yields (as per family average) in spring. For example, the average seed yield of family 13B6 at HE was 0.426 g in spring 2009, whereas 10A1 and 10B1 produced no seeds whatsoever - they die before they could flower because of onset of dry weather (Table 5-3-2 HE).

GH	2007		2008				2009	
	Autumn		Spring		Autumn		Spring	
	Seed yield (g)	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*
1B5	0.449	0.025	0.547	0.084	0.426	0.068	0.933	0.069
1D1	0.435	0.052	0.778	0.043	0.432	0.041	0.660	0.039
2C2	0.565	0.016	0.848	0.111	0.652	0.030	0.823	0.042
2E1	0.475	0.060	0.664	0.130	0.755	0.027	1.084	0.076
2E5	0.507	0.043	0.966	0.094	0.522	0.086	1.139	0.047
4A4	0.438	0.051	0.599	0.075	0.483	0.057	0.887	0.088
5A3	0.319	0.015	0.408	0.110	0.376	0.057	0.867	0.075
6A3	0.378	0.020	0.299	0.088	0.611	0.044	1.117	0.073
7B5	0.508	0.042	0.987	0.059	0.660	0.094	1.073	0.072
8A3	0.388	0.024	0.612	0.058	0.503	0.164	-	-
8A4	0.398	0.035	0.652	0.168	0.646	0.092	1.009	0.061
9A2	0.406	0.059	0.853	0.017	0.477	0.043	0.919	0.070
10A1	0.472	0.091	0.267	0.016	0.347	0.083	0.252	0.025
10B1	0.408	0.087	0.207	0.086	0.701	0.049	0.265	0.068
11A2	0.510	0.048	0.789	0.079	0.452	0.056	0.730	0.089
11A5	0.512	0.145	0.248	0.102	0.530	0.029	0.680	0.054
11B2	-	-	-	-	0.333	0.034	0.734	0.073
12A1	-	-	0.192	0.033	0.294	0.033	1.028	0.037
13A2	-	-	-	-	0.661	0.064	0.977	0.074
13B6	0.613	0.020	0.535	0.101	0.545	0.070	1.104	0.054

Table 5-3-2 **Average family seed yield (g) of selected families in autumn and spring from 2007 to 2009 (1 of 3)**.

LE	2007		2008				2009			
	Autumn		Spring		Autumn		Spring		Autumn	
	Seed yield (g)	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*
1B5	0.055	0.000	0.143	0.057	0.034	0.003	0.450	0.050	0.165	0.020
1D1	0.049	0.008	0.125	0.013	0.043	0.023	0.512	0.087	0.164	0.010
2C2	0.009	0.000	0.197	0.056	0.030	0.006	0.305	0.039	0.187	0.015
2E1	0.031	0.018	0.166	0.050	0.036	0.003	0.391	0.059	0.208	0.015
2E5	0.028	0.003	0.245	0.069	0.032	0.004	0.413	0.078	0.199	0.009
4A4	-	-	0.068	0.028	0.025	0.003	0.215	0.026	0.130	0.008
5A3	0.051	0.019	0.074	0.021	0.027	0.006	0.397	0.099	0.225	0.015
6A3	0.018	0.003	-	-	-	-	0.397	0.063	0.169	0.012
7B5	0.018	0.006	0.104	0.023	0.044	0.003	0.487	0.033	0.131	0.012
8A3	0.029	0.010	-	-	-	-	-	-	0.109	0.007
8A4	0.021	0.006	0.175	0.039	0.040	0.003	0.411	0.026	0.149	0.013
9A2	0.018	0.004	0.088	0.021	0.037	0.005	0.288	0.040	0.113	0.006
10A1	-	-	0.033	0.016	0.030	0.003	0.166	0.031	0.221	0.009
10B1	0.035	0.009	0.160	0.048	0.048	0.005	0.089	0.035	0.276	0.006
11A2	0.009	0.002	0.072	0.037	0.032	0.003	0.421	0.045	0.206	0.013
11A5	0.008	0.002	0.073	0.017	0.029	0.004	0.543	0.056	0.198	0.018
11B2	0.019	0.005	0.152	0.041	0.030	0.003	0.450	0.047	0.210	0.019
12A1	-	-	0.049	0.030	0.043	0.004	0.246	0.050	0.151	0.014
13A2	0.047	0.013	0.095	0.024	0.044	0.005	0.446	0.054	0.188	0.015
13B6	-	-	0.039	0.025	0.030	0.007	0.388	0.080	0.238	0.022

Table 5-3-2 **Average family seed yield (g) of selected families in autumn and spring from 2007 to 2009 (2 of 3)**.

HE	2007		2008				2009			
	Autumn		Spring		Autumn		Spring		Autumn	
	Seed yield (g)	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*	*Seed yield (g)*	*SE*
1B5	0.049	0.009	0.107	0.015	0.022	0.006	0.264	0.021	0.059	0.005
1D1	0.044	0.010	0.063	0.013	0.010	0.002	0.328	0.023	0.043	0.008
2C2	0.069	0.010	0.088	0.016	0.018	0.003	0.265	0.037	0.089	0.014
2E1	0.090	0.023	0.124	0.013	0.014	0.002	0.318	0.043	0.085	0.010
2E5	0.065	0.007	0.101	0.013	0.030	0.011	0.293	0.037	0.063	0.013
4A4	-	-	0.070	0.013	-	-	0.181	0.024	0.072	0.007
5A3	0.052	0.007	0.127	0.014	0.021	0.003	0.226	0.029	0.098	0.015
6A3	0.054	0.011	-	-			0.218	0.029	0.076	0.010
7B5	0.044	0.006	0.114	0.016	0.019	0.002	0.329	0.022	0.049	0.009
8A3	0.050	0.009	-	-	-	-	-	-	0.068	0.010
8A4	0.036	0.002	0.074	0.023	0.025	0.008	0.300	0.049	0.082	0.011
9A2	0.039	0.009	0.041	0.007	0.023	0.004	0.168	0.023	0.078	0.014
10A1	-	-	0.140	0.015	0.029	0.005	0.000	-	0.070	0.008
10B1	0.087	0.013	0.174	0.019	0.051	0.011	0.000	-	0.135	0.014
11A2	0.070	0.008	0.095	0.008	0.026	0.004	0.237	0.021	0.082	0.017
11A5	0.058	0.010	0.092	0.010	-	-	0.272	0.000	0.080	0.007
11B2	0.070	0.009	0.099	0.012	0.041	0.005	0.236	0.021	0.084	0.017
12A1	0.069	0.012	0.105	0.016	0.029	0.004	0.266	0.018	0.072	0.013
13A2	0.072	0.018	0.133	0.078	0.047	0.004	0.294	0.022	0.075	0.008
13B6	-	-	0.137	0.008	0.028	0.003	0.426	0.086	0.062	0.010

Table 5-3-2 **Average family seed yield (g) of selected families in autumn and spring from 2007 to 2009 (3 of 3)**. Late flowering families at HE, such as 10A1 and 10B1, did not make it to reproductive stage in 2009. Keys: **GH**, unheated unlit greenhouse; **LE**, low elevation; **HE**, high elevation; ***SE***, standard error; "-", not grown for the season.

Heritability of seed yield

Heritability (H^2) was estimated using all seasonal seed yield data of selected families from LE and HE. The number of individuals forming each family involved in H^2 calculation ranged from two to twelve, as a consequence of variation in the number of plants that survived to fruit. The resultant H^2 values varied among seasons – the majority was found to be low, but unexpectedly high for a few seasons (ranging from 0.53 to 0.61, Table 5-3-3). The low H^2 values are unsurprising as seed production can be strongly influenced by environmental factors, in particular, the resources availability within a season at the time of fruiting. Conversely, the high H^2 values suggest that seed yield variation is highly genetically determined under field conditions. This can be seen as consistent with adaptation to the environment; that is, families with genetically preserved adaptations (to the environment and/or local seasonal conditions) would be expected to produce greater seed yield in the conditions to which they were adapted.

In addition, ANOVA indicated that the seed yield differed significantly between the 20 selected families in most of the experiments (Table 5-3-4). The only exception came from the set of seeds of LE autumn 2008, where seed yield differences between families were insignificant.

	2007	**2008**		**2009**	
	Autumn	**Spring**	**Autumn**	**Spring**	**Autumn**
LE	0.15	0.07	-	0.53	0.61
HE	0.25	0.15	0.31	0.59	0.17

Table 5-3-3 **Summary of heritability (H^2) estimated from seed yield of 20 selected families at LE and HE**. Most seed yields of field grown local *A. thaliana* populations in seasonal experiments have low H^2, which could be attributable to environmental factors. Keys: **LE**, low elevation and **HE**, high elevation. Keys: "-", not applicable due to insignificant difference.

	2007	2008		2009	
	Autumn	**Spring**	**Autumn**	**Spring**	**Autumn**
LE	$F_{14,51} = 2.196$ $p = 0.02$	$F_{17,71} = 2.71$ $p = 0.002$	$F_{17,175} = 1.039$ $p = 0.42$	$F_{18,107} = 4.586$ $p = 2.6 \times 10^{-7}$	$F_{19,157} = 10.62$ $p = 9.6 \times 10^{-20}$
HE	$F_{16,77} = 1.994$ $p = 0.02$	$F_{17,86} = 1.927$ $p = 0.03$	$F_{15,163} = 4.034$ $p = 3.2 \times 10^{-6}$	$F_{16,73} = 8.564$ $p = 3.5 \times 10^{-11}$	$F_{19,146} = 2.709$ $p = 0.0004$

Table 5-3-4 **One-way ANOVA from all seasonal seed yield of 20 selected families at LE and HE**. All collected seeds from selected families grown within the season were compared. The differences of seasonal seed yield at LE and HE are significant. Keys: **LE**, low elevation and **HE**, high elevation.

Seasonal seed yield contribution from selected families

The recorded seed yields of all surviving individuals from the selected families in an experiments were used to determine the percentage contribution of that individual to total seed yield in the experiment (*individual percentage contribution = (individual seed yield / total seed yield of all selected families)*100*).

To gain an insight into the overall performance of selected families, the average contribution per family member was calculated (*family average = sum of individual percentage contributions / number of individuals in family*). This was necessary because the number of family members could differ within experiments. This value was then normalized for each experiment, using the sum of all average family contributions (*family contribution = (family average/ sum of all family averages)*100*); giving a relative contribution of an average family member compared to the average contributions of members of other families (Fig. 5-3-1). Seed yield contribution provided an estimate of relative fitness; families with individuals contributing a greater percentage of the overall seed yield can be said to be fitter than those families contributing a lower percentage.

Figure 5-3-1 shows the varying average percentage contributions of the 20 selected families from GH, LE and HE sites in four consecutive seasons. In autumn, the average seed contribution from each family grown in GH ranged from 2.9% to 8.0%. Families

such as 5A3 (2007) and 12A1 (2008) were amongst the lowest contributors, whereas 13B6 (2007) and 2E1 (2008) were the highest.

Autumn field plants showed a wide range of individual contributions (Fig. 5-3-1 LE & HE). For example, the average seed contribution at LE ranged from 1.9% (11A5) to 12.6% (1B5) in 2007; 3.8% (4A4) to 7.5% (10B1) in 2008; and 3.0% (8A3, 9A2) to 7.6% (10B1) in 2009 (autumn 2009 data are not included in Fig. 5-3-1). At HE, the average seed contribution was between 3.5% (8A4) and 8.9% (2E1, 10B1) in 2007; 2.5% (1D1) and 11.8% (10B1) in 2008; and 2.9% (1D1) and 9.1% (10B1) in 2009 (autumn 2009 data not shown in Figure). The family with the lowest percentage seed yield is seen to vary across the seasons and years recorded; the only exception was 1D1 which made the lowest contribution at HE in both autumn 2008 and autumn 2009. In contrast, the highest seed contributor was relatively consistent; 10 B1 made the highest contribution for five out of the six season and location combinations (the exception being autumn 2007 at LE, where 1B5 contributed the greatest percentage).

In spring, the familial contribution at GH varied in a range between approximately 1.3% and 7.0%. Families 12A1, 10A1 and 10B1 were the lowest contributors, with 7B5 and 2E5 being the greatest. Families10A1 and 10B1 were amongst the lowest contributors for both springs, being joined by 12A1 in 2008. Family 2E5 showed similar consistency in being amongst the greatest contributors for both years (joined by 7B5 in 2008). Interestingly, when grown in GH, family 7B5 produced a similar seed contribution (approximately 6.6%) in all recorded autumn and spring seasons.

Field plants at LE from spring experiments contributed a greater range of percentage seed yield than their GH cohorts. The range of average percentage contribution for families was between 0.7% (13B6) and 15.0% (2E5) in spring 2008, and between 1.2% (10B1) and 7.6% (11A5) in spring 2009. A wider range was also observed from HE plants; the average spring seed contribution at HE was between 2.2% (9A2) and 9.3% (10B1) in 2008, and between 0% (10A1 and 10B1) and 9.2% (13B6) in 2009.

Although contributions could be seen to vary within and between experiments, a common pattern could be observed for differences in the relative contributions of some families. Such patterns were either seasonal or reflect the geographic origin of families. Firstly, *seasonally based* patterns are seen where there are consistent differences in the contribution of a family between seasons at all locations. Secondly, *origin based* patterns occur where families from the same locale show similar relative contributions at the same experiment site and season.

The examples for *seasonally based* patterns are families 10A1, 10B1 and 13A2, which were among the lowest contributors in spring experiments. In particular, the seed contribution differences between autumn and spring from family 10B1 were significant ($p = 0.008$ for autumn 2007/ spring 2008; $p < 0.001$ for both autumn 2008/ spring 2009 and spring 2009/ autumn 2009; autumn 2009 data are not included in Fig. 5-3-1). Family 13A2, was also observed to consistently produce more seeds during autumn than spring in at least two of the experimental sites (LE and HE; there is insufficient data for GH from autumn 2007and spring 2008, though autumn 2008/ spring 2009 shows the same pattern as LE and HE; Fig. 5-3-1).

O*rigin based* patterns: Examples of this pattern can be found in families from locations 2 (2C2, 2E1 and 2E5) and 11 (11A2, 11A5 and 11B2, at HE in particular). When evaluating autumn 2007/ spring 2008, all families from location 2 contributed less seeds in autumn than in spring at LE, but more seeds autumn than in spring at HE. Group 11 showed a similar patterns; the majority of members contributed less seed in autumn than in spring at LE (except 11A2), but more seeds in autumn than spring at HE (Fig. 5-3-1 LE & HE).

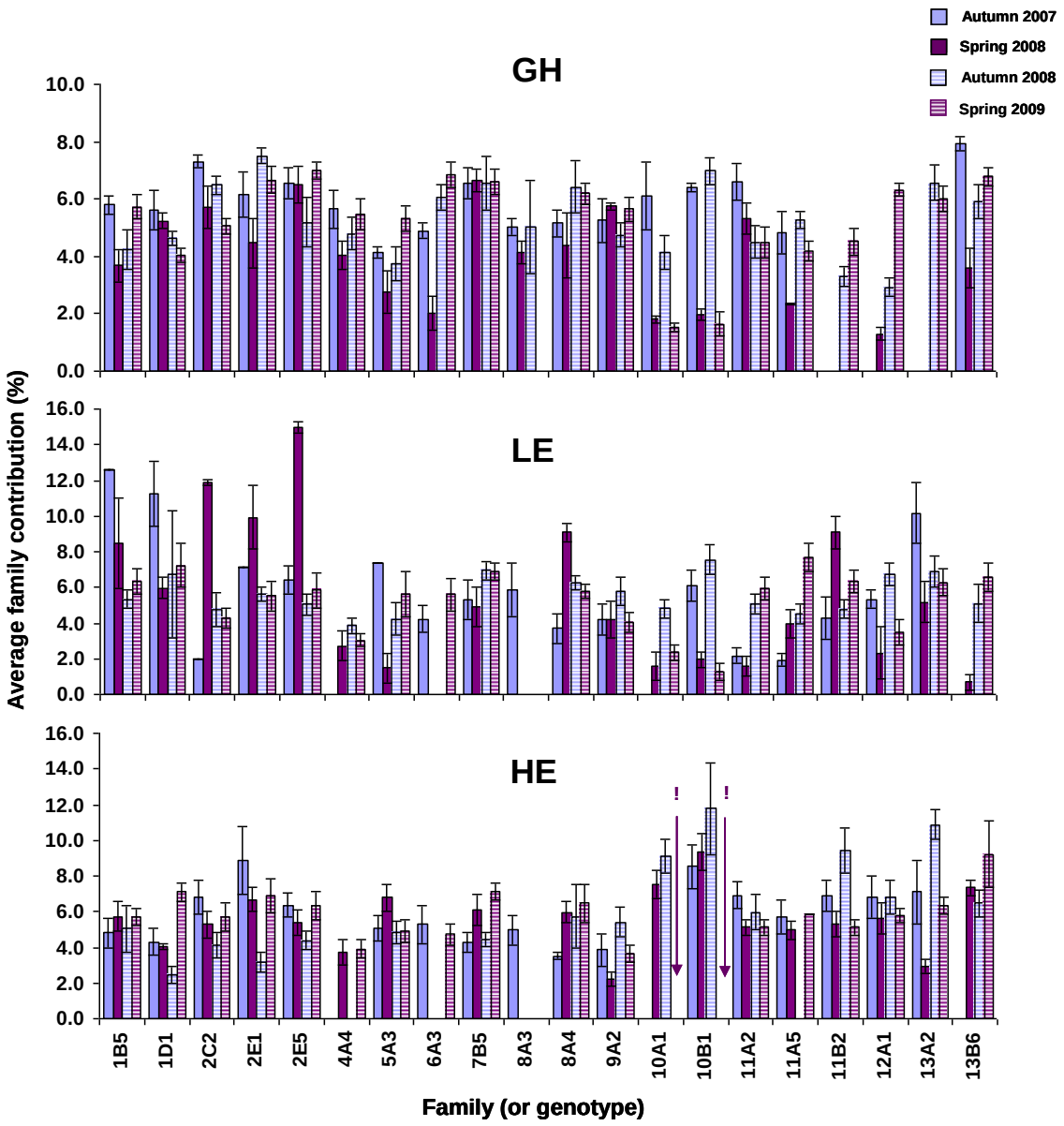

Figure 5-3-1 **The average family seed contribution from 20 selected families**. The average contributions from each family to the total seed yield within an experiment are plotted for different sites and seasons, from autumn 2007 to spring 2009. **GH**, unheated unlit greenhouse; **LE**, low elevation; **HE**, high elevation; "**!**", none of the individual survived to fruit; blank column represent families not included in the experiment due to low germination. Values are family means with bars representing standard errors.

Seed Size

Variation in seed size can potentially affect estimates of fitness. On one hand, larger seeds could lead to total seed yield over-estimating the potential number of offspring. On the other hand larger seeds could be more likely to improve the chances of seedling establishment and survival, while smaller seeds exhibit superior advantage of colonization due to the higher dispersal.

In order to investigate variation in seed size, seeds from six experiments (autumn and spring sets and both LE and GH) were examined. A random sample of 100 seeds were counted for each plant and then weighed. One-way ANOVA indicated significant differences in average individual seed mass between families in five of the six experiments. Individual seed mass ranged approximately between 1.5 µg and 2.5 µg.

Seed mass was significantly different between seasons, in five out of six sets of comparison (i.e. LE or GH in combination of autumn 08 versus spring 08, autumn 08 versus spring 09 and spring 08 versus spring 08). It is expected that plants could potentially produce slightly larger or smaller seeds depending on seasonal conditions. The seed mass differences between seasons were less (and insignificant in one of three comparisons) at GH, presumably because growing conditions within the facility were less variable than the natural environment at LE.

In addition, there was no correlation between individual seed size and total seed yield – i.e., families that produced *heavier* seeds were not necessarily ones that produced the least *total* seed yield - and vice versa. Due to the relative consistency of individual seed mass, and the difficulty in estimating the effects of seed size on fitness, seed size was not factored into fitness estimates.

The relationship between seed yield and growth in autumn and spring

Total seed yields from the 20 selected families were plotted against an estimate of rosette growth rate (area), to determine the association between these two traits. A weak but significant positive correlation between average family growth and seed yield was observed in most autumn experiments: faster growing individuals tended to produce more seeds than those that grew slower. The strength and significance of correlations was also observed to vary from autumn of different years, depending on weather conditions at the start of the experiment. In general, the milder the weather at start, the stronger and more significant the correlation between average growth and seed yield, i.e. autumn 2009 > 2007 > 2008.

An example of the relationship between average family growth and its average total seed yield from LE autumn 2008 is shown in Figure 5-3-2A, where $r = 0.29$ and $p = 0.25$. LE autumn 2007 showed a similar but significant correlation ($r = 0.34$, $p = 0.04$, data not shown). Growth and seed yield in autumn 2009, however, was both highly significant as well as strongly correlated ($r = 0.78$, $p = 0.0001$; Fig. 5-3-2B). This is probably because plants were better established (i.e. bigger rosette areas in general, compared to cohorts from previous autumns) due to milder weather (Chapter 4) at the start of the growth period.

Similarly, HE autumn 2007 and 2008 showed a weak but positive correlation between average family growth and seed yield ($r = 0.38$, $p = 0.02$ and $r = 0.19$, $p = 0.48$, respectively; data not shown), whereas autumn 2009 cohorts showed a stronger and highly significant correlation with $r = 0.70$ ($p = 0.002$). In GH, the seed yield and growth are also weakly but positively correlated (i.e. autumn 2007 gave r-value of 0.29 with $p = 0.0005$). This suggests that faster growing individuals have a better chance of producing more offspring than slower growing ones in autumn.

Unlike autumn populations, average growth was not correlated with seed yield in most spring experiments. Figure 5-3-2C shows an example of the relationship between

average family growth and its average total seed yield from LE spring 2009 population (r = -0.28, p = 0.20). A similar lack of correlation was also observed in both LE and HE spring 2008, where r = 0.18, p = 0.15 and r = -0.05, p = 0.67 respectively. An interesting exception was that at GH in spring 2008 and 2009, where seed yield was negatively correlated with growth (r = -0.28, p = 0.04 and r = -0.38, p = 0.0002, respectively). A similar negative correlation was also recorded in HE spring 2009 (r = -0.68, p = 0.02). These negative correlations were independent of the fast-growing but late-flowering. However, by removing the data for fast-growing but late-flowering families in HE spring 2009 population (family 5A3 and all families from location 10), the significant correlation was lost (p = 0.18) and the strength of negative correlation weakened (r = -0.35).

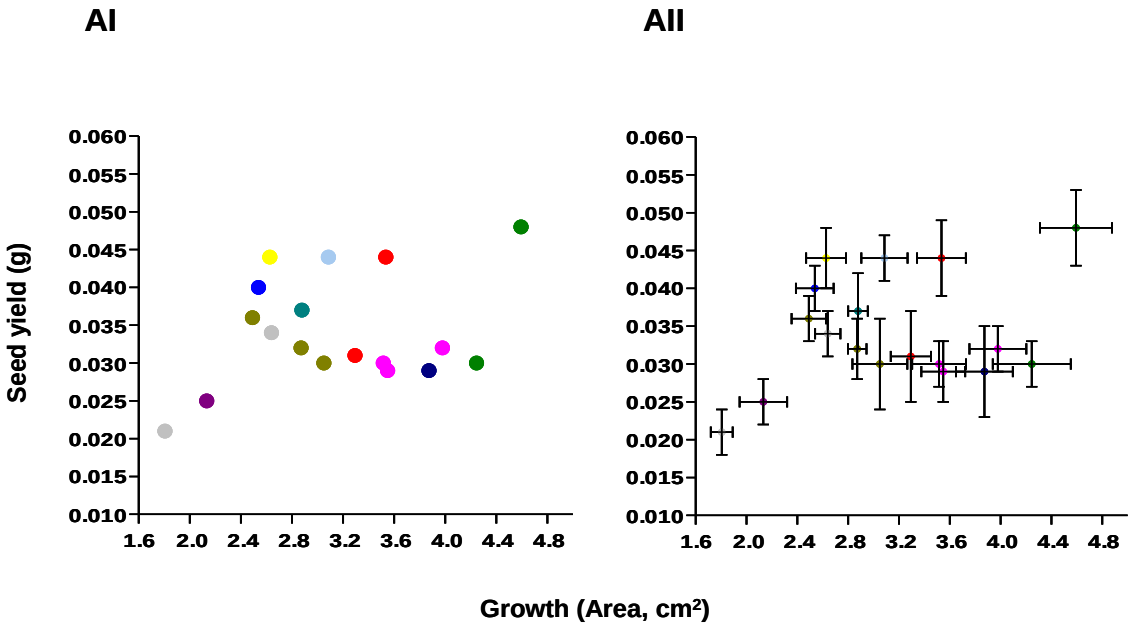

Figure 5-3-2 **The relationships between seed yield and growth in autumn 2008 (A), 2009 (B) and spring 2009 (C) at LE (1 of 2)**.

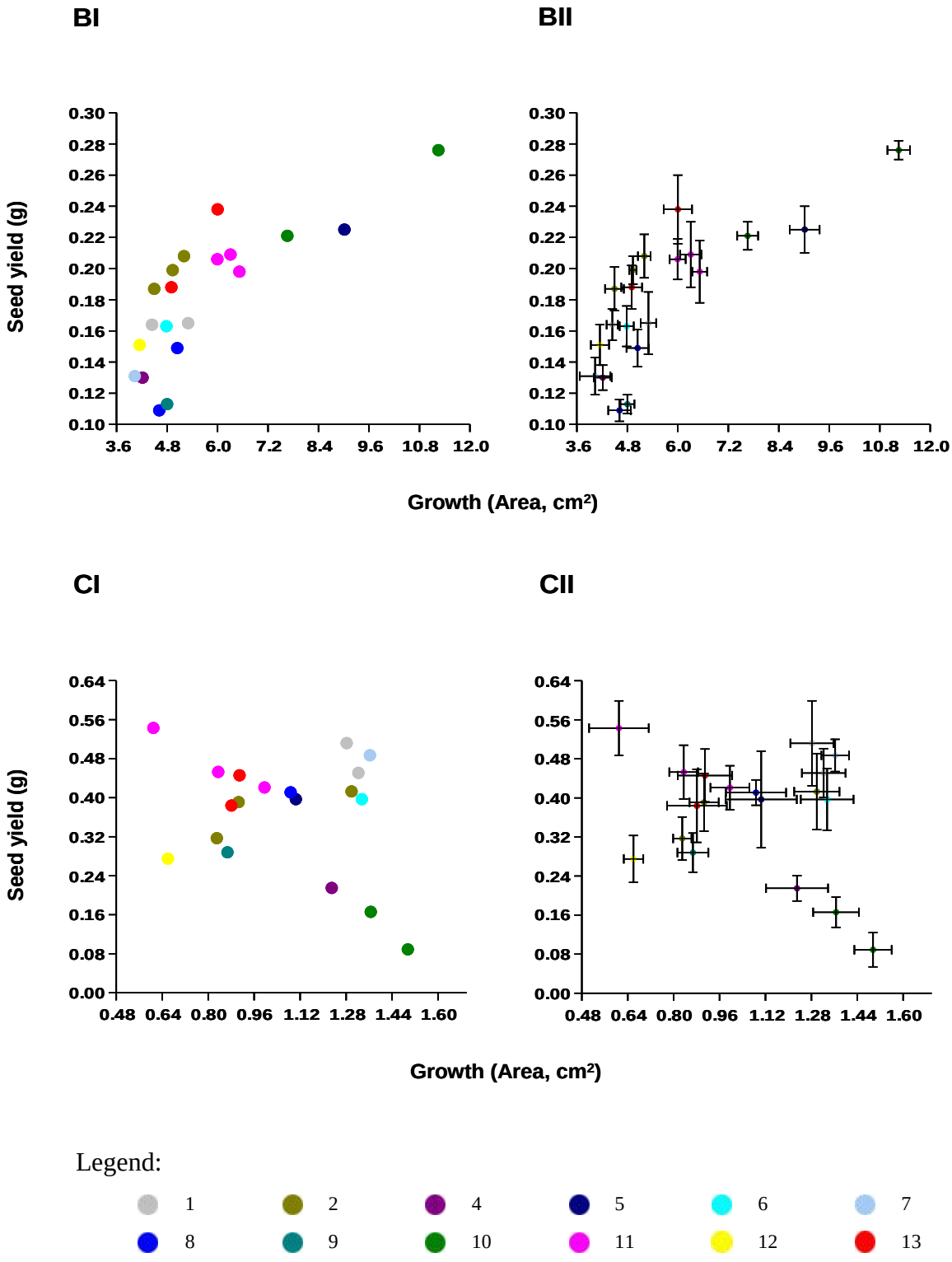

Figure 5-3-2 **The relationships between seed yield and growth in autumn 2008 (A), 2009 (B) and spring 2009 (C) at LE (2 of 2)**. A positive correlation between seed yield and growth was observed in autumn (**A**: r = 0.29, p = 0.25; **B**: r = 0.78, p =

0.0001), whilst a negative (or sometimes lack of correlation) in spring (**C**: r = -0.28, p = 0.20). Family mean are shown and colour coded based upon the sampling locations 1 to 13 of the local accessions (**I**) with ± *SE* (**II**). Key: dsg = days since germination.

The relationship between seed yield and bolting in autumn and spring

Average family seed yield and bolting (or flowering) time were correlated in a few, but not all, autumn sets of plants. For example, seed yield and bolting time at LE were observed to be weakly correlated in autumn 2009; both the seed/ bolting and seed/ flowering correlations were determined to be significant (bolting, r = 0.59, p = 0.007; flowering, r = 0.50, p = 0.03 respectively; Fig 5-3-3 A & B). Under these conditions, later flowering plants tended to produce more seeds than earlier flowering ones.

Plants from LE autumn 2008, however, were not seen to have any significant correlation between their bolting time and seed yield (r < 0.01, p = 0.99), or between flowering time and seed yield (r = 0.02, p = 0.95). Similar lack of correlation between bolting and seed yield was also recorded for GH population in the same season (r = 0.09, p = 0.15). Nonetheless, a weak but significant positive relationship was recorded between flowering and seed yield for population at HE (r = 0.45, p = 0.08) in autumn 2008.

In contrast to autumn cohorts, spring populations were observed to have a weakly negative correlation between average family seed yield and bolting time (i.e., earlier flowering plants tended to produce more seeds). This can be seen in Figure 5-3-3C, which shows the relationship between bolting time and total seed yield in the LE spring 2009 population (r = -0.33, p = 0.2). This relationship, however, is dependent on the inclusion of the very late flowering families from location 10. After removing these individuals, the correlation became positive and significant (r = 0.48, p = 0.05).

Both populations at GH in spring 2008 and 2009 also showed highly significant negative correlations (r = -0.56, p < 0.0001 and r = -0.59, p < 0.0001, respectively). This strongly

suggests that bolting (or flowering) time may have a substantial influence on total seed yield in spring.

The results of growth rate and bolting/ flowering time variation from Chapter 5-2 indicated that the correlations of these two traits were mostly depended on the outliers, i.e. the extreme early or late flowering families, suggested the relationships between spring growth and bolting required climate maintenance. For example, bolting/ flowering in GH were observed to be slightly more mediated by weather changes in spring than in autumn. This further suggests, at least for spring populations, that differences in timing of flowering may be a more important component of fitness than differences in growth rate.

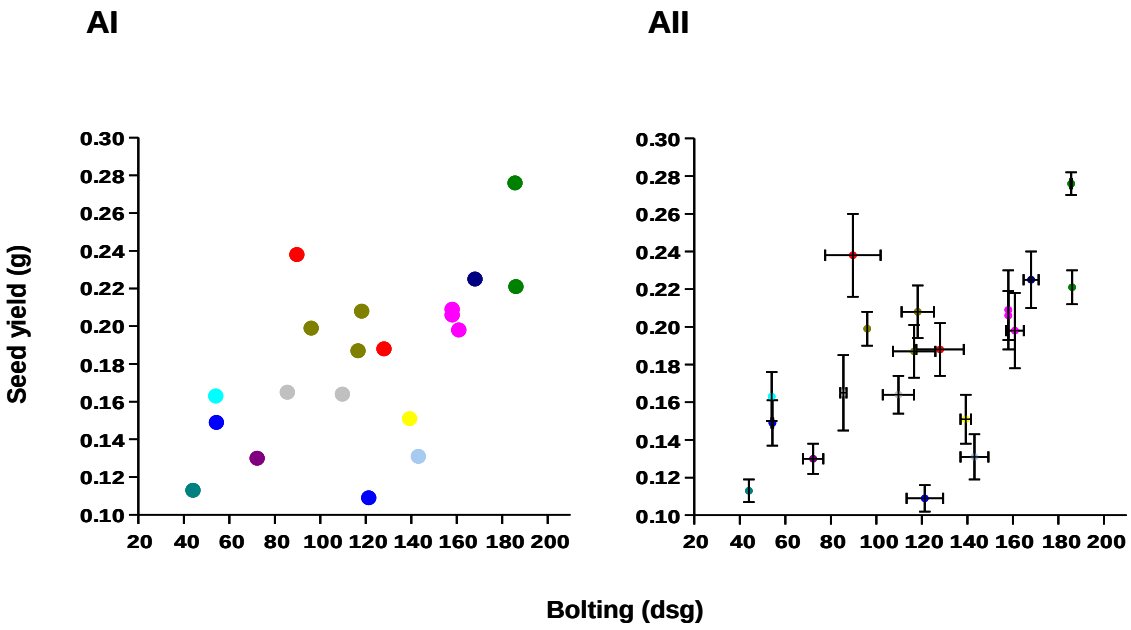

Figure 5-3-3 **The relationships between seed yield and bolting time, seed yield and flowering time at LE in autumn and spring 2009 (1 of 2)**.

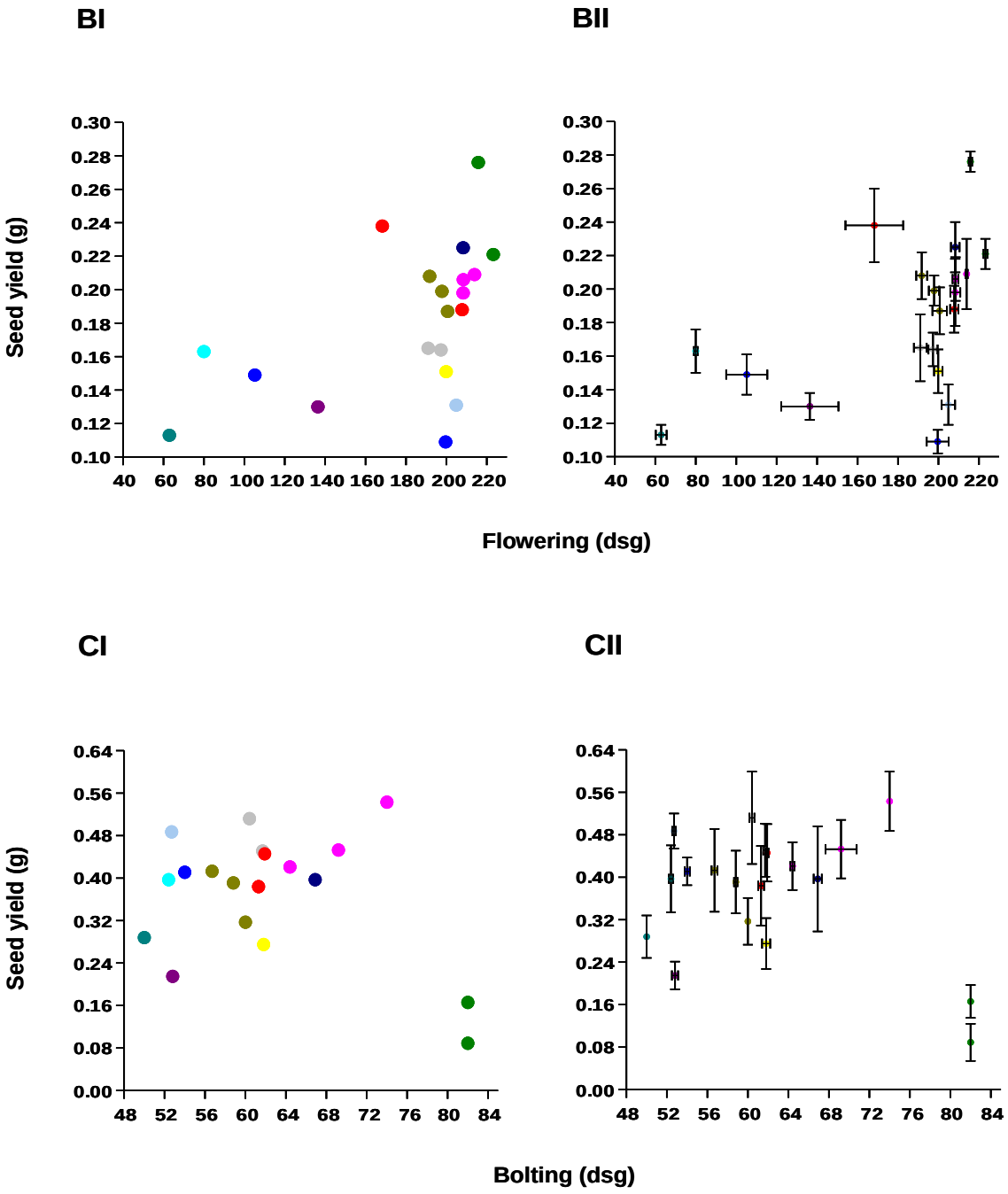

Figure 5-3-3 **The relationships between seed yield and bolting time, seed yield and flowering time at LE in autumn and spring 2009 (2 of 2)**. There is a weakly but significant correlation between autumn average family seed yield and bolting (r = 0.59, p = 0.007; **A**), as well as flowering (r = 0.50, p = 0.03; **B**). In spring however, the correlation was insignificant (r = -0.33, p = 0.2; **C**). Family mean are shown and colour coded based upon the sampling locations 1 to 13 (see legend on p121 for colour code reference) of the local accessions (**I**) with ± *SE* (**II**). Key: dsg = days since germination. Key: dsg = days since germination.

Different growth stages and total seed yield

Different growth stages from LE autumn and spring 2009 were investigated to explain total seed production. Table 5-3-5 shows the r^2 values for autumn rosette growth between 15 and 60 dsg against total seed yield. All growth stages in autumn are significantly correlated with seed yield, where the correlations strengthened with age in general. This means that the older the plants were, the better they served as estimates of total seed yield. In addition, coefficient of variation, CV [(standard deviation/ mean)*100] shows that the highest correlations between growth stage and seed yield also has the highest CV. This suggests that r^2 does not correlate with the relative amount of variance at each time point. Although both growth and seed yield were highly heritable in spring (Table 5-1-1A & 5-3-3), there was no significant correlations between various growth stages and total seed yield.

Area (dsg)	CV	F	r^2
15	28.42497	4	0.02
25	27.41433	90	0.34
28	27.96174	57	0.25
32	31.39036	53	0.24
35	30.77507	67	0.28
39	33.44462	88	0.38
43	34.53082	101	0.37
53	38.36452	131	0.43
60	40.45171	147	0.46

Table 5-3-5 **Different growth stages (in dsg) and seed yield of LE autumn 2009 population**. The correlation between growth and seed yield increases with age. F = F-statistics.

Discussion

Seed mass and number are frequently used to estimate fitness. Variation in the number of fruits, seeds per fruit and seed mass will contribute to differences in total yield between individuals. Trade-offs between yield components (i.e. fruit size and seed per fruit) however, may buffer total yield; increases in fruit number may lead to a decrease in seeds per fruit (Winn and Werner 1987; Marshall *et al*. 1985). Hence, an increase in fruit number will not necessarily lead to variation in total yield. In this chapter, total seed yield was used rather than seed number as an estimate of fitness due to relatively little variation in seed size between genotypes (refer section seed size, p118), as well as the relative simplicity and ease of measurement.

Seed number was, nonetheless, estimated using weight of 100 seed and total yield from an autumn and spring season (where most individuals per family were counted). Each 100 seed weight was divided by total yield (in grams) and multiplied by 100 to obtain seed number estimate. Figure 5-3-4 shows that seed number estimates for LE autumn 2008 and spring 2009 are well correlated with total yield, indicating that the greater seed number, the greater the total yield.

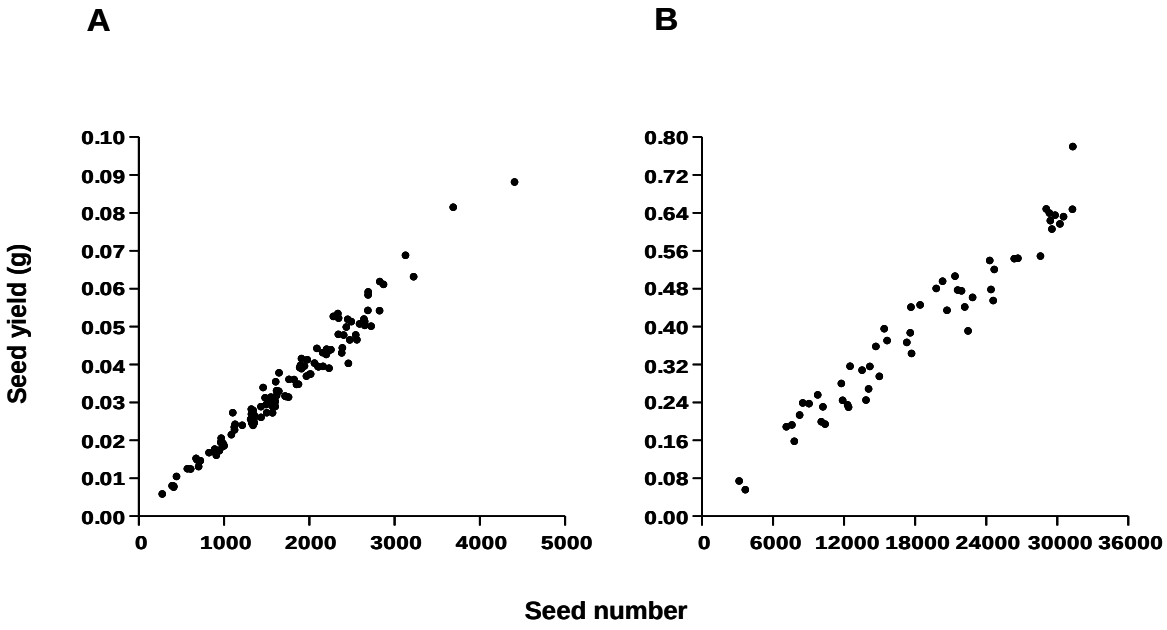

Figure 5-3-4 **The relationships between seed yield and seed number in autumn 2008 (A), and spring 2009 (B) from LE**. Between five and seven individuals per family were selected in random order. A random sample of 100 seeds per individual was then counted and weighed. R-values for autumn and spring are 0.98 ($p < 0.0001$) and 0.97 ($p < 0.0001$), respectively.

Total seed yield in different environmental conditions

Total seed yield was observed to be influenced by environmental conditions, as evidenced through plants producing more seeds at GH compared to LE and HE. This outcome can be explained through the sheltered conditions providing a more optimal growth environment (i.e. warmer temperatures and reduced herbivore predation). The marginal seed yield differences between LE and HE within a season suggested environmental differences between the sites were too low to significantly impact yield.

A similar explanation could apply to the observed seed yield difference between autumn and spring (for LE and HE especially); weather conditions in most springs were more

optimal for growth. When autumn conditions were spring-like in 2009, the total population seed yield was higher than other autumns.

Plant survival and seed yield

Factors noted to impact LE and HE sites during experimental periods include animal predation (i.e. slugs, pigeons and rabbits in autumn and spring), human damage (from building construction work in near LE site) and early summer drought. The observed pigeon and rabbit predation showed no preference towards particular families and simply ate the accessible individuals. Seasonal climate factors were observed to have a more pronounced mortality impact (with specific regards to the very late flowering genotypes in spring) compared to animal or human factors. Otherwise, the typical mortality rate of local genotypes during seasonal experiments was low and random.

At LE, for example, spring 2008 was a particularly bad period in terms of individual mortality due to non-seasonal climate factors; plants suffered from slugs and pigeons, as well as destroyed by workers due to building construction nearby experimental site at around 30 dsg. A total of 62 plants (n = 519; at least one replicate per family out of approximately two thirds of the families) incurred various degrees of leaf damage from nibbling or tearing by herbivory; 41 of these survived to fruit. In addition, the construction incident destroyed 6 plants. In the same spring, rabbits instead of pigeons were noted to consume *A. thaliana* at HE, along with slugs. Around 70 plants consumed by rabbits and 15 by slugs (as identified by the nature of damage) were recorded. Although the average family seed yield from LE and HE spring 2008 were less than their 2009 cohorts, they generally produced a greater yield than their autumn cohorts (Table 5-3-2).

A more substantial mortality rate, in this case attributable to climate, was observed in late flowering genotypes in dry spring/ early summer in 2009 - those very late flowering families at HE, predominant from sampling location 10, died of drought. Over half of LE

cohorts in the same spring, however, survived but were among those that produced the lowest seed yield within the population. Another very late flowering genotype, family 5A3, also suffered increased mortality in dry spring but less severely compared to the 10s. Some individuals of 5A3 survived to fruit at HE; all survived and fruited at LE.

Potential seasonal variables responsible for seed yield variation

A strong association was found between weather – specifically the variables of temperature and rainfall - and total seed yield and different variables were more important in different seasons. Autumn 2009 was warmer than previous years (Chapter 4). In autumn 2009, total seed yield from the experiment was higher than previous autumns (Table 5-3-1), suggesting a relationship between warmer temperature and higher seed yield. The final rosette sizes of field-grown plants was also larger on average in autumn 2009 compared to the previous two autumns and similar to that observed in spring. Suggesting that warmer temperature (combined with the timing of rainfall) promotes vegetative growth, which can in turn affect seed yield. Flowering time can also affect seed yield in autumn experiments, presumably because it affects the duration of vegetative growth. This can be seen in the correlations between flowering time and seed yields in autumn experiments and is illustrated by late flowering families such as 10B1, 11B2 and 13A2, which are among the greatest seed producers (10B1 contributed 7.6% of the seeds produced by the selected families in autumn 2009) compared to the early flowering like 9A2 and 8A4 families, which only contributed approximately 3.0% each.

For spring plants, rainfall had a more obvious effect upon seed yield, particularly at the time when siliques were developing. Each year a short dry period of approximately two to three weeks was observed at the end of spring. The inflorescences of late flowering plants rarely survived to fruit as a consequence. One particularly serious environmental impact upon seed yield was observed in spring 2009, when, the majority of individuals from families 5A3, 10A1, 10B1 and 12A1 at HE produced no seeds. These families were late flowering and their vegetative growth stretched into a short period of drier weather

(with approximately 50 mm of average rainfall per month from April to June, late flowering families typically bolt around May/ June). All individuals from families 10A1 and 10B1 died before seed set. Only the minority (42%) of individuals from family 5A3 at HE were able to produce seeds and their yields were lower than their LE cohorts, so although they survived the short period of drought, seed yield was severely affected.

Individual family seed yield variation between seasons

Several families were found to show superior fitness in one season compared to the other, consistently producing significantly more seeds during the preferred season. The seasonal seed yield differences strongly suggest that these individual families may be adaptive to a particular season. Evidence for local adaptation to factors that vary geographically has been found for members of the same or different species (Volis 2002, 2007; Li *et al*. 2002; Reviewed by Jump and Peñuelas 2005). Seasonal fitness variation may arise from local adaptive differentiation due to population size from various sampling sites. A similar natural variation has been documented in other selfing annual. An early study on natural *Impatiens pallida* (Yellow Jewelweed) populations (sampled from two forests approximately 50 km apart) found that the populations had maintained significant genetic variation (Schemske 1984). Schemske scored a number of quantitative traits which including flowering time and seed production, and demonstrated local adaptation at one of the two original forest localities in a reciprocal transplant experiment. This work also found significant genetic variation in local *Impatiens pallida*, both between transects and among-families within transects. It was further suggested that spatial scale is particularly important in selfing species, because a small effective population size could increase the potential for local differentiation.

However, the results in this study are interesting in showing seasonal preference in local *A. thaliana* from similar geographic locations within a 5 km radius. Weather data (Chapter 4) showed only minor differences between experimental sites. Although the HE site was around 2°C cooler than the LE site on average, this temperature difference is

much less than occurs between seasons. Therefore adaptation to different seasons can explain the variation observed in local populations better than adaptation to factors that vary geographically within 5 km.

Such differentiation could occur because genotypes that were fitter in autumn produced more seeds to be subsequently deposited into soil, as would those spring-fit genotypes. The resultant seed bank would thus contain the seeds produced from both winter and spring adapted populations.

A. thaliana seeds were shown to retain the ability to germinate across seasons for over two years (Olatunde Akinola *et al*. 1998; Baskin and Baskin 1983; Roberts 1968). Some *A. thaliana* seeds were also found to be non-dormant and germinate in the field over a wide range of temperatures (Baskin and Baskin 1983). Baskin and Baskin (1983) suggested that seeds from some natural *A. thaliana* populations may not induce into complete dormancy during the cold season due to insufficient period of vernalization, inappropriate temperature during mild winter or simply buried too deep in the soil. In a quick test for natural germination, most seeds (a few batches from different seasonal collections) sown onto potting soil in autumn 2008 at LE germinated (personal observation). This suggests both groups (winter and summer adapted genotypes) germinated during the autumn prior to when local populations were sampled for this study. This is consistent with the parent plants having been collected from the field in late January and February.

The observed *origin based* similarities in seed yield between families can be attributed to the relatedness of families from the same locality (Chapter 3) and do not necessarily provide evidence for local adaptation to factors that vary geographically. However, there may be an interaction between geography and seasons – for example, some sites might experience more disturbances in one season that allows germination or seedling establishment – and so genotypes from one location might be predominantly autumn or spring adapted.

Although for many families (e.g. families from location 2) the seed yield differences between seasons were not significant, similarities in seed yield contribution patterns (as seen in Fig. 5-3-1) suggest some might be adapted to local conditions that vary geographically. It may be hypothesized that these families have developed similar mechanisms for responding to particular sets of environmental variables. However, further research would be required to isolate and quantify the role of individual abiotic (or biotic) influences.

The significance of growth (RGR) on seed yield

Different traits, i.e. growth and flowering time, were found to have different influences on fitness in different season. Fast growth (greater RGR) was observed to be advantageous trait in autumn. This is supported by the positive correlations between relative growth rate and seed yield observed in most autumns. The benefit of faster growth in autumn may be due to the deterioration of weather conditions towards the end of the year. In most winters growth became negligible during the months of December to February, presumably because of low temperatures, freezing and low irradiance and photoperiod. When conditions for growth improved in spring, most plants flowered within a short time of each other. Therefore, plants built up their resources mainly through vegetative growth in the autumn.

In spring, however, fast growth was observed to be mostly neutral (lacking correlation to seed yield) or disadvantageous (with a negative correlation to seed yield). A fast growth strategy may even be potentially redundant during spring if it serves a primary purpose of maximizing resources in scarce periods (i.e. winter). Importantly, faster growth may incur greater risk than slower growth during spring; larger plants are more susceptible to stresses such as predation and, because they transpire more (to drought). They might also be more susceptible to irradiance (higher UV exposure), herbivory, or the effects of high temperature. In the case of herbivory and irradiation, plants which are larger are more obvious targets for predation and have a larger surface area exposed to irradiance with a

reduced chance of canopy coverage from neighbouring plants. These increased risks may lead to the observed negative correlations seen between seed yield and growth in some spring populations.

Another possible explanation for the contrasting correlations between growth and seed yield in autumn and spring, at least for GH populations growing under more controlled conditions, is that there was greater variation between the growth of families in autumn than in spring (while bolting remained similar both seasons). The GH populations consistently showed higher coefficient of variation for autumn growth than spring (approximately 36% to 47% in autumn versus 24% in spring). The lower growth variation in spring populations was likely arose as all plants were able to grow close to an optimal rate. Therefore growth rate might have had only a minor, undetectable effect on differences in seed yields between families.

The significance of bolting/ flowering time on seed yield

In this study, bolting and flowering times were observed to have a different level of impact on seed yield depending on the season. Seed yield showed a significant positive correlation with flowering or bolting time in autumn. However, seed production from plants that flowered early from an autumn sowing was likely to be compromised by severe winter weather, especially if plants flowered before the onset of winter (i.e. autumn/ winter 2009). Late flowering, however, could be seen to contribute to higher fitness in autumn or spring - but only if flowering occurred before drought conditions in early summer. Late flowering families, if they could set seed prior to drought (as suggested by the seed/ flowering correlation from LE spring 2009 population), were fitter than early flowering plants (a positive contribution between flowering time and seed yield was seen when the extremely late flowering families were excluded). In addition, the late flowering phenotype from autumn sown plants was observed to be mostly neutral (lacking correlation with seed yield) when growth conditions were mild (i.e. at GH, and

LE in autumn 2008) or beneficial (positively correlated with seed yield), over colder winter (i.e. LE autumn 2009).

A late flowering trait could hypothetically evolve because of benefits stemming from a prolonged growth period, which allow more use of available resources and thus improve fitness (greater seed yield due to larger plants at maturity). Furthermore, late flowering plants would potentially be able to take advantage of more optimal conditions in mid spring (as a result of being in vegetative phase rather than flowering phase, throughout the harsher winter months) which would also act to help increase seed yield.

It was observed that late flowering autumn plants typically flowered in mid spring - which was abundant in resources such as rainfall and day length. This would reduce resource-related pressures, reducing the effective benefits of late flowering trait. However, general risk factors associated with the late flowering trait - such as mortality due to drought, predation and depletion of nutrients - also increase with time. This risk increase would explain why late flowering individuals would not necessarily have the same increased fitness associated with late flowering under different climate conditions (i.e. where weather varied over the same seasons in different years) with contingent different levels of risk.

In summary, bolting/ flowering time was a more influential trait upon fitness in spring. Spring populations showed more variation in the association between flowering time and seed yield; there was a positive correlation between flowering time and seed yield up to a certain time point. When families flowering after this point were included, a negative correlation was observed – this may indicate that these individuals were flowering at a disadvantageous time period. Families flowering later, but before this time period, were likely benefitting from increasingly optimal conditions; such as by avoiding early-summer droughts whilst benefitting from increasing day length.

Summary Conclusion

Data suggested that phenotypes, growth rate and bolting/ flowering time, have different advantages in different seasons. Fast growth is better when resources are scarce and later flowering (but not flowering too late) is better when resources are abundance.

The difference between the influence of flowering time upon seed yield in autumn and spring is interesting in evaluating the importance of the trait. It is suggestive that, bolting/flowering time is not an indicator of overall fitness during those time periods where growth rate has been observed as an important trait in influencing seed yield.

Chapter 6 Competition between seasonal fast and slow *A. thaliana*

Introduction

All living organisms are forced to engage in competition in order to ensure access to resources (both for sustenance and reproductive purposes), and thus survival. It is an inevitable consequence that this competition influences population dynamics (Cheplick, 1992); applying both to natural and agricultural plant communities. Competition in plants can take many forms; strategies include aerial (shading light from reaching neighbouring vegetation), or underground (removing water or nutrients from the soil or releasing chemicals that deter other plant species). One well known study documents the production of potent chemicals by *Ailanthus altissima* (commonly known as 'tree of heaven') that inhibit the seedlings of many species from germinating (Heisey 1990). In addition to possessing this potent ability to poison rivals, *A. altissimas'* competitive arsenal includes growing at an extraordinarily rapid rate: allowing it to both shade competitors and reach the reproductive stage earlier than most other tree species.

Investigation of the effects of competition has examined different growth conditions, such as levels of soil nutrients or plant density. A large number of early investigations focused on root competition, due to its greater effects on the plant (root growth, root density and root surface area) and because competition below ground can involve as many, or more, neighbours than competition above the ground (reviewed by Casper 1997). In more recent years, studies have focused on the mechanism of interactions between plants via chemical and physiological components (i.e. root exudates and hormonal signaling; de Kroon 2007; Dudley and File 2007; Gruntman and Novoplansky 2004).

Research by Willis *et al*. (2010) suggested that genetic relatedness is important in competition tolerance. This study evaluated the potential involvement of genetic variation in competitive tolerance and neighbour suppression. RILs were generated by crossing two homozygous *Arabidopsis thaliana* accessions (Columbia x an unknown

genotype): most loci of these RILs were expected to be homozygous, yet collectively to possess a greater range of allelic variation than either of the single parental populations alone. The RILs were thus considered to resemble selfing *A.thaliana* found in the wild.

Target plants were chosen from the highest and lowest quintile for vegetative size: these were paired in turn with a competitor from the same genotype or from a different RIL (not one of the target genotypes). Competition was found to reduce the fitness (seed production) of all genotypes and the ability of a target plant to tolerate competition was affected by its own genotype and the genotype of its competitor. The fitness of a target plants was also suppressed less by a neighbour that had a different genotype, than one that had the same genotype.

A number of plant behaviours have been documented in response to competition (Novoplansky 2009), such as avoidance (growing away from shade or expected shade, as seen in shoots) (reviewed by Franklin 2008), confrontation (behaviour aimed at impeding the development of competitors) or tolerance (behaviour to maximize performance under the conditions caused by competing neighbour) (Novoplansky 2009; Callaway 2002; Casper 1997). These behaviours are not totally exclusive, and plants may adopt a combination of several as their competitive strategy. In most of these studies, the outcomes provide substantial evidence that prediction of plant growth performance under competition is possible under certain growth conditions.

This chapter is focused on growth performance of selected local genotypes grown in competition under natural conditions. Very little of the surveyed literature investigating competition has examined wild *Arabidopsis thaliana* under natural seasonal conditions. The life cycle of *A. thaliana* allows the comparison of growth across two different seasons; either in autumn as a biennial or summer as an annual. As a model organism, understanding the competitive responses of *A. thaliana* would benefit the general understanding of plant competition.

The growth outcome from competitive experiments could bring some insight into the involvement of competition in adaptation. Results from Chapter 5 suggested that growth performance differences between local genotypes were genetically determined. The competitive experiments in this chapter aimed to investigate the predictability of growth, in competition, for genotypes selected on the basis of their genetically determined growth differences in isolation. However, these experiments were not designed to determine the degree to which genetic variation affects competition, nor the precise mechanisms involved.

Result

Growth data presented in the previous chapter (5) showed that some local genotypes growth performance was consistent with season: many families performed well in one particular season and poorly in the other (Table 6-1). However, these experiments involved plants growing in isolation from each other and with other *A. thaliana* plants, as is likely to occur in the wild. In order to investigate how competition might affect the growth and seed yields of plants in different environments, experiments were set up at LE and HE sites for autumn and spring. The experiments also involved plants growing in isolation, to further test whether the previous responses of families to different seasons could be used to predict their performance.

Families were paired based on their previous years' performances; fast growing families were selected for competition against slow growers (Table 6-1). Three pairs of families were selected: 8A4 (autumn slow, spring fast) was paired with 11B2 (autumn fast, spring slow); 4A4 (autumn slow, spring fast) with 13B6 (autumn fast, spring slow) and finally 10B1 (autumn fast, spring fast) with 12A1 (autumn slow, spring slow). Members of these families were planted in pairs; individuals of each family faced competition from either an individual of the alternate family (i.e. 8A4 versus 11B2) and from their own siblings (i.e. two 8A4 individuals or two 11B2 individuals in each pot). For further

comparison, solitary individuals were planted for each family to give a baseline of performance without competition (Fig. 6-1).

	Autumn	Spring
8A4 *(pair 1)*	slow	fast
11B2 *(pair 1)*	fast	slow
4A4 *(pair 2)*	slow	fast
13B6 *(pair 2)*	fast	slow
10B1 *(pair 3)*	fast	fast
12A1 *(pair 3)*	slow	slow

Table 6-1 **Growth performances of different families in autumn and spring** Genotypes were selected according to their growth performances in previous years. *Pairs 1*, *2* and *3* represent competition pairing for growth comparison, i.e. 8A4 is paired with 11B2.

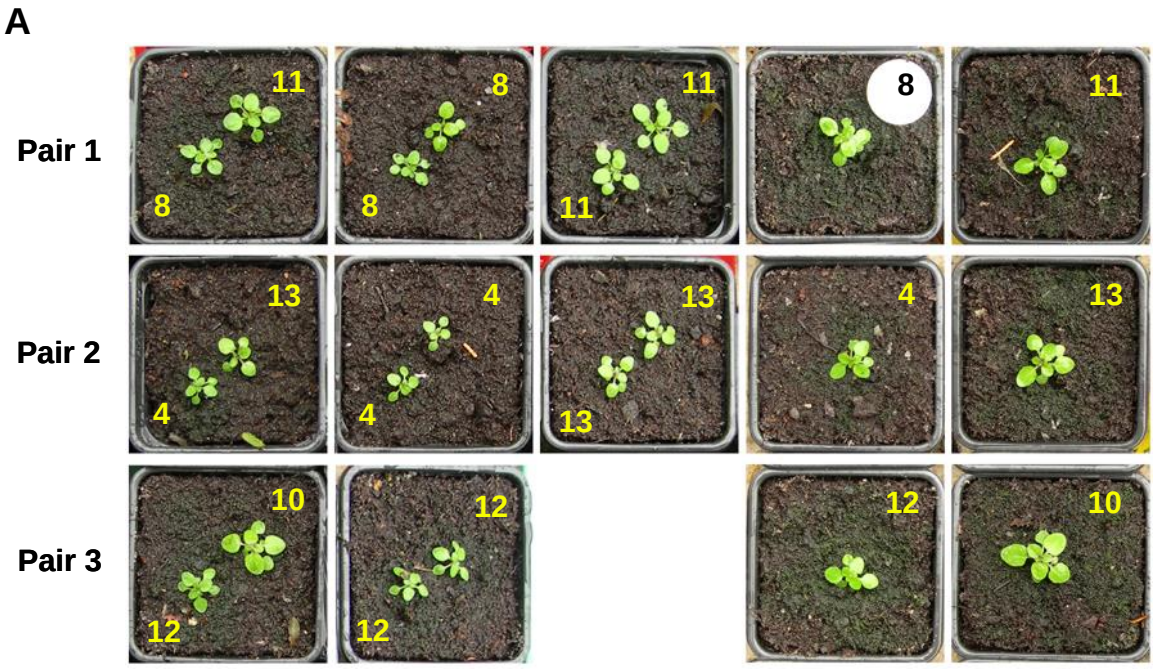

Figure 6-1 **An illustration of seasonal growth competition (1 of 2)**.

Figure 6-1 **An illustration of seasonal growth competition (2 of 2)**. Different competition pairings and solitary plants grew in autumn (**A**) and spring (**B**) at LE. Each pair of plants (8A4 vs 11B2, 4A4 vs 13B6 and 10B1 vs 12A1) was grown with 2.5 cm space between the competing individuals. Petiole length and leaf blade morphology of competing and solitary plants were observed to be similar. Keys: **Pair 1** consists of 8A4 (**8**) and 11B2 (**11**); **Pair 2**, 4A4 (**4**) and 13B6 (**13**); **Pair 3**,10B1 (**10**) and 12A1 (**12**); white circle is the area reference.

Growth and competition

I. Autumn

In autumn, solitary plants (-nc) without competition generally grew faster (were larger at sampling time) at LE than HE (Fig. 6-1A & 6-2), as had been observed before. The relative performance of families was also generally consistent with the results of previous field studies. The three families that had been the fastest growing in previous autumns (10B1, 11B2 and 13B6) were the fastest in this experiment at both HE and LE. The families that were paired in competition experiments had significantly different growth

rates from each other in isolation. Solitary plants from pairings 8A4 versus 11B2, 4A4 versus 13B6, and 10B1 versus 12A1 differ significantly in their growth rates at LE ($p = 0.01$, $p = 0.001$ and $p < 0.0001$, respectively) and more so at HE ($p = 0.001$, $p = 0.0001$ and $p < 0.0001$). Despite a slight growth rate differences between LE and HE solitary plants, the growth correlation at the two elevations remained high ($r = 0.98$).

The growth rate of all families that were tested in self (or sibling, -sc) competition decreased at both elevations (Fig. 6-2). Different genotypes responded differently to self competition. For example, 11B2 and 13B6 had similar solitary growth rates, but the growth rate of 13B6 was reduced more by self competition at LE (at LE; it was not tested at HE). Similarly, 11B2 grew significantly faster than 12A1 at HE without competition but had a similar growth that to 12A1 in self-competition. Although different families responded differently to self-competition the rank order of growth rates of the families remained approximately the same as for solitary plants.

Non-self (or non-sibling, -c) competition reduced the growth rate of all families relative to solitary plants at HE. At LE it reduced the growth rates of all families significantly, except 8A4 and 11B2. The effects of non-self competition were similar to those of self-competition for most families. These did not show a significant difference in growth rate between the two types of competition. The exceptions were that 4A4 and 12A1 at LE and 11B2 at HE grew faster in non-self competition than when competing against themselves. This is consistent with more competition occurring between genetically identical individuals. More importantly, there was a correlation between the growth of families in isolation and in non-self competition. This suggests that the relative performance of a family in competition can be predicted from it performance as a solitary individual.

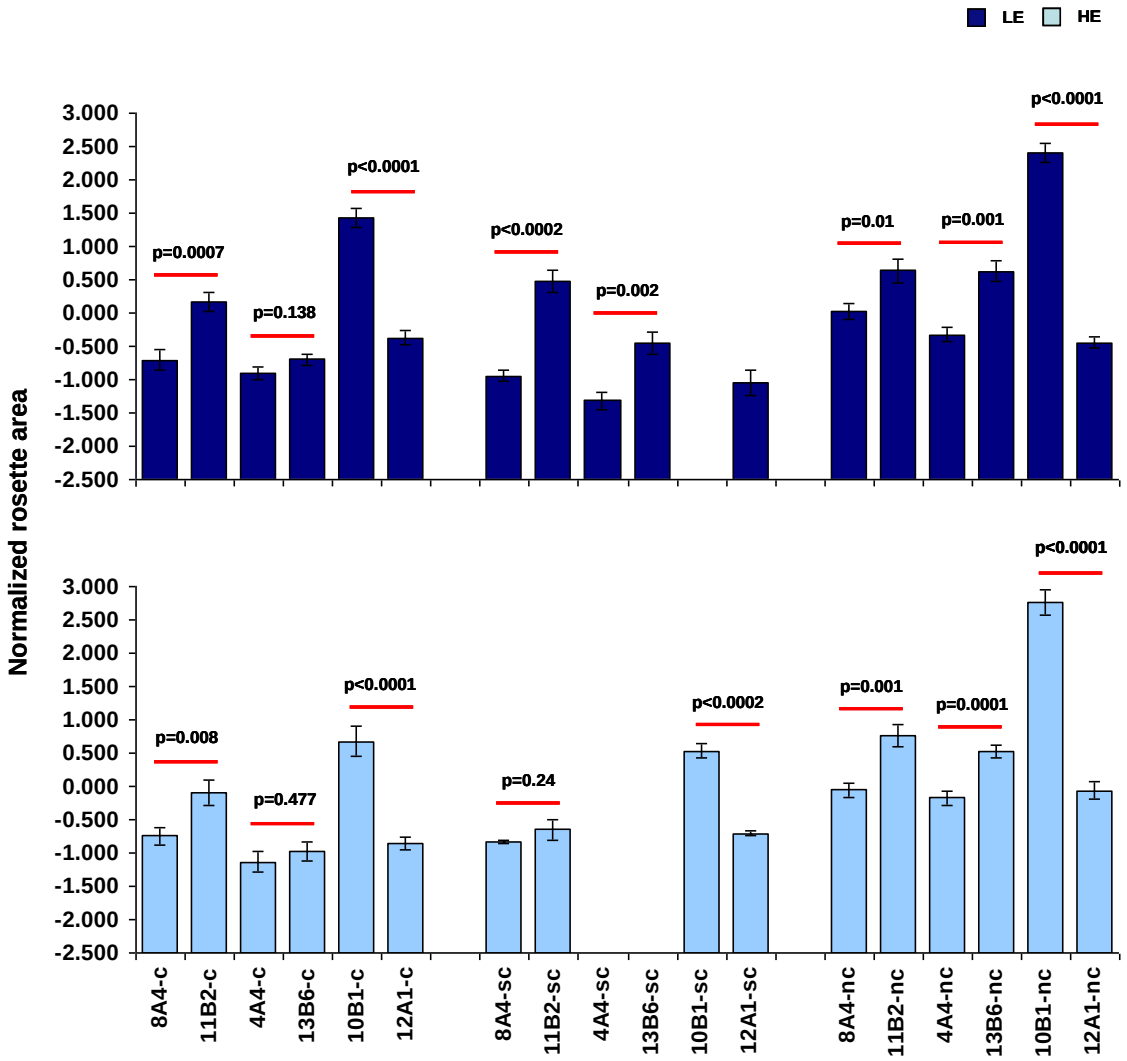

Figure 6-2 **Autumn growth competition**. Growth of each individual was estimated with digital aerial photography and then values normalized within an experiment. Mean values are shown with their standard errors for six to eight members of each family. P-values for significance of differences in growth rate between pairs of are presented. Individual genotype is labeled on the x-axis. Keys: **-c** denotes competition with non-sibling, **-sc** is sibling competition, **-nc** is a solitary plant.

II. Spring

In spring, most families grown as solitary individuals (-nc) differed in their growth rates with the exception of 8A4 and 11B2 at LE and HE, which were seen to grow at similar

rates to each other (Fig. 6-1B & 6-3). In addition, the growth correlation between LE and HE remained strong ($r = 0.83$).

Unlike autumn cohorts, the growth rate of all families in self (-sc) competition increased at both elevations (Fig. 6-3). However, different genotypes responded differently to self competition at the two elevations. The difference between the pairs of plants selected for fast or slow growth in spring was greater at HE than LE and highly significant. This has altered the growth rank order slightly from solitary cohorts.

There was some growth difference between fast and slow growing families, but it was observed in non-sibling competition such as 4A4 and 13B6 (Fig. 6-3). The differences in growth rate within this pair of individuals were greater at HE than at LE. This slightly weakened the correlation between LE and HE to $r = 0.63$ for this non-sibling competition group.

Non-self (-c) competition also augmented the growth rate of all families relative to solitary plants at both LE and HE. This is the opposite outcome compared to autumn cohorts. In spring, it increased the growth rates of all families significantly. The effects of non-self competition were similar to those of self-competition for most families, except 11B2 at HE.

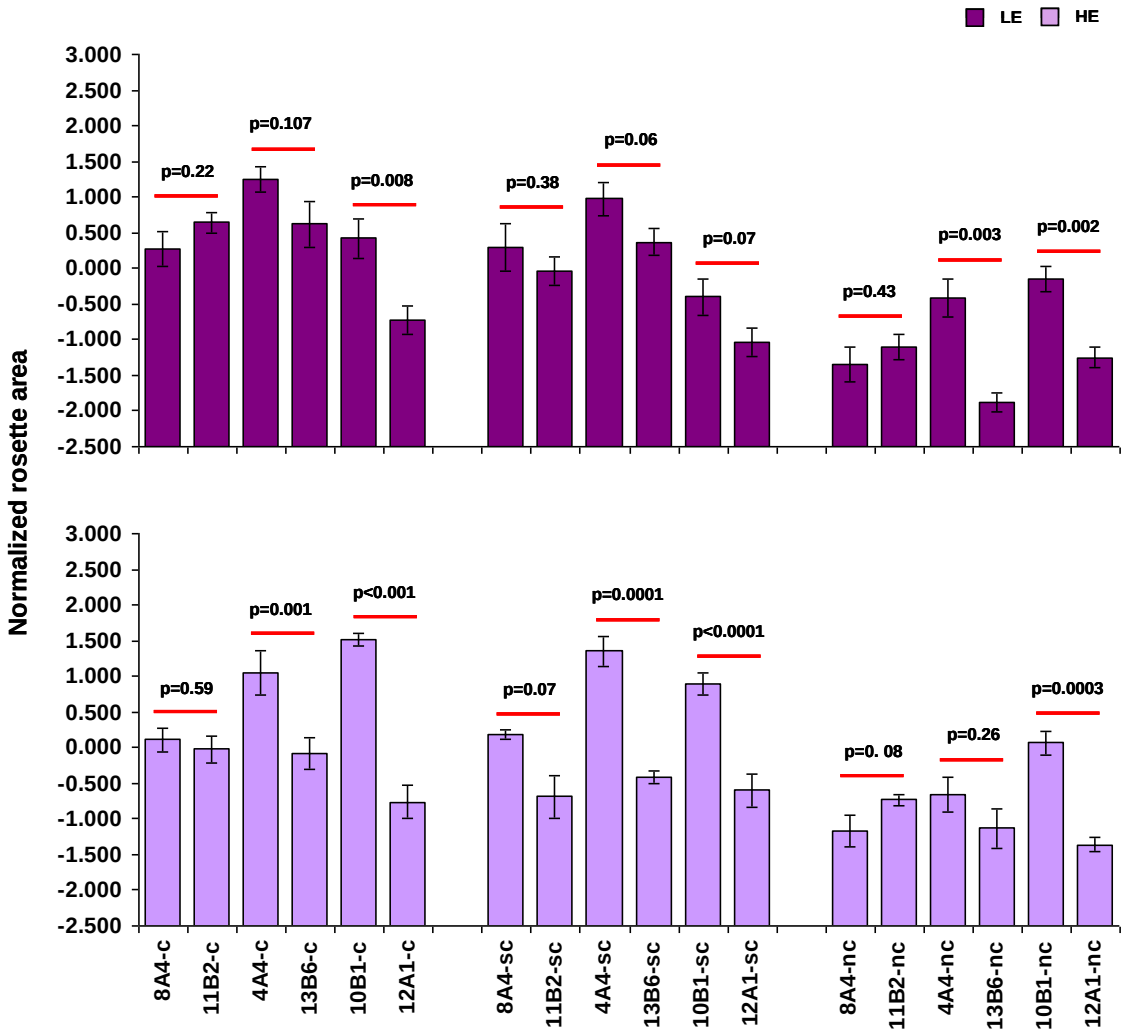

Figure 6-3 **Spring growth competition**. Data are presented as in Fig. 6-2.

Growth differences with or without a competitor

The growth of plants with competitors was significantly slower than for solitary plants in autumn (Fig. 6-2 & Table 6-2A). For example, families 8A4, 4A4 and 13B6 in competition grew significantly slower than in the solitary cohorts at both LE ($p = 0.001$, 0.01 and 0.001) and HE ($p = 0.0005$, 0.0002 and 0.0002). Interestingly, the identity of competitors was not observed to alter the growth of most competing genotypes. The marginal growth differences observed between competition with non-sibling and sibling (c : sc) were insignificant for the majority of comparisons (Table 6-2A).

In contrast, competition in spring was observed to have an opposite effect on competing individuals (Fig. 6-3 & Table 6-2B). Considering again the families 8A4, 4A4 and 13B6 that grew slower when in competition in autumn, individuals grew significantly faster in competiton (with siblings or non-siblings) in spring at both LE and HE. This suggests that autumn and spring had different influences on the interactions of the plants with their competitors.

In addition, Table 6-2C shows that the marginal growth differences between elevations for most competing families (with sibling or non-sibling) were observed to be insignificant. The exceptions, 10B1 and 11B2, were seen to have significant growth differences at different elevations when in competition. This suggests that competition can interact with elevation to affect growth rate.

A	**Autumn**					
	LE			**HE**		
Genotype	***nc : c***	***nc : sc***	***c : sc***	***nc : c***	***nc : sc***	***c : sc***
8A4	0.001 $^{+}$	<0.0001 $^{+}$ *	0.25	0.0005 $^{+}$ *	0.0003 $^{+}$ *	0.51
11B2	0.07	0.54	0.20	0.003 $^{+}$	0.0002 $^{+}$ *	0.04
4A4	0.01	0.001 $^{+}$	0.02	0.0002 $^{+}$ *	-	-
13B6	0.001 $^{+}$	0.006	0.18	0.0002 $^{+}$ *	-	-
10B1	0.001 $^{+}$	-	-	0.0002 $^{+}$ *	0.0002 $^{+}$ *	0.57
12A1	0.63	0.004	0.01	0.001 $^{+}$	0.003 $^{+}$	0.17

B	**Spring**					
	LE			**HE**		
Genotype	***nc : c***	***nc : sc***	***c : sc***	***nc : c***	***nc : sc***	***c : sc***
8A4	0.002 $^{+}$	0.006	0.96	0.001 $^{+}$	0.001 $^{+}$	0.65
11B2	0.0002 $^{+}$ *	0.004	0.02	0.01	0.91	0.096
4A4	0.0002 $^{+}$ *	0.003	0.37	0.005	0.0001 $^{+}$ *	0.42
13B6	0.0004 $^{+}$ *	0.0002 $^{+}$ *	0.52	0.03	0.03	0.29
10B1	0.15	0.50	0.05	0.0002 $^{+}$ *	0.005	0.01
12A1	0.09	0.49	0.28	0.06	0.04	0.65

Table 6-2 **P-values for differences between solitary and competitive growth (1 of 2)**.

C	Autumn		Spring	
	LE ***c*** : HE ***c***	LE ***sc*** : HE ***sc***	LE ***c*** : HE ***c***	LE ***sc*** : HE ***sc***
8A4	0.09	0.05	0.62	0.83
11B2	0.03	<0.001 + *	0.01	0.01
4A4	0.01	-	0.58	0.24
13B6	0.87	-	0.10	0.008
10B1	0.004 +	-	0.005	0.004 +
12A1	0.003 +	-	0.91	0.01

Table 6-2 **P-values for differences between solitary and competitive growth (2 of 2)**. Values are shown for autumn (**A**), spring (**B**) and different elevations (**C**). P-values were estimated from *t*-test using PAST. Correlations that remained significant at p = 0.05 and p = 0.01 after Bonferroni correction are shown by + and *.
LE and **HE** are low and high elevation sites respectively; ***nc : c*** denotes comparison between solitary growth and non-sibling competition cohort, ***nc : sc*** is comparison between solitary growth and sibling competition cohort; **LE** or **HE : *c*** or ***sc*** is comparison between non-sibling competition at LE and HE, or comparison between sibling competition at LE and HE.

Seed yield from competitive growth

I. Autumn

Solitary plants (-nc) that were grown at both LE and HE produced more seeds than plants in competition (Fig 6-4). At LE, seed yield from the autumn fast growing families (11B2, 13B6 and 10B1) was significantly more compared to the paired slow growing families (p = 0.02, p = 0.006 and p < 0.0001, respectively). Most fast growing families grown in isolation at HE produced less seed than LE cohorts. Unlike LE, the differences of seed yield between fast and slow families growing in isolation were mostly insignificant at HE.

Seed yield in all families decreased in self (or sibling, -sc) competition at both elevations. Nonetheless, autumn fast growing families produced more seeds than the slow growing families at LE and HE. For example, the seed yield difference of 8A4 and 11B2 were indistinguishable when each was grown separately at HE, but 11B2 was observed to

produce more seed than 8A4 in self competition. This similar trend of seed yield difference (with better significance) was observed in the same pair at LE.

The effects on seed yields of non-self competition (or non-sibling, -c) were similar to those of self-competition for most families. Seed yield differences between the fast and slow members of all three competing pairs (8A4 versus 11B2, 4A4 versus 13B6 and 10B1 versus 12A1) were significant at LE ($p < 0.0001$, $p < 0.01$ and $p = 0.04$). At HE, most seed yield differences between competitor pairs were insignificant except 10B1 versus 12A1 ($p = 0.006$).

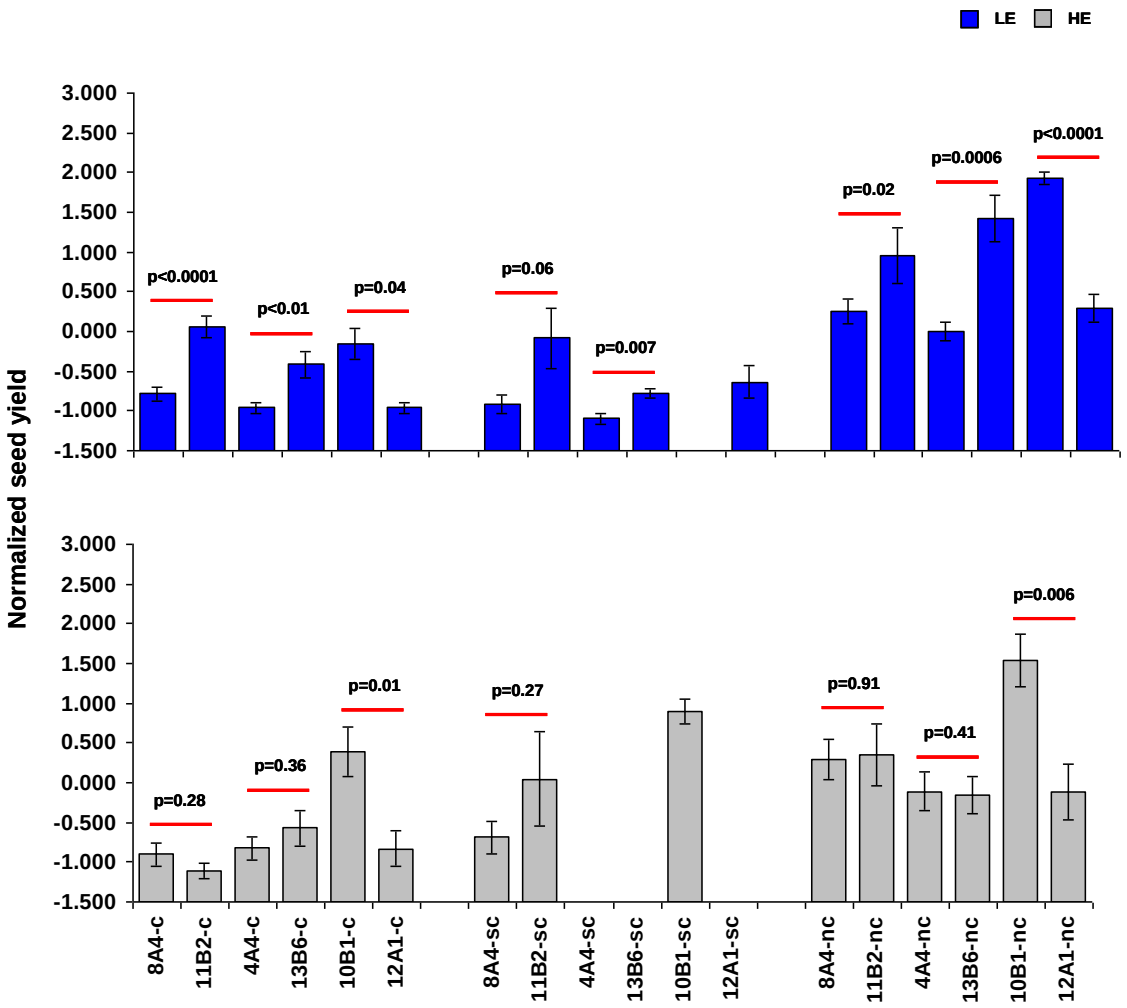

Figure 6-4 **Autumn seed yields from solitary and competing individuals**. Seeds were harvested at maturity then the weight produced by individuals was normalized within an experiment. Mean values are shown with their standard errors for six to eight members of each family. P-values for significance of differences in yield between pairs of are presented. Individual genotype is labeled on the x-axis. Keys: **-c** denotes competition with non-sibling, **-sc** is sibling competition, **-nc** is a solitary plant

II. Spring

In spring, most families (both solitary and in competition) produced different seed yield (Fig. 6-5; no seed collection from HE). Families that grew faster in spring, such as 8A4 and 4A4, produced greater seed yield than slow growing families. In particular, solitary

plants from fast families 8A4 and 4A4 produced significantly more seeds than slow 11B2 and 13B6 ($p = 0.04$, $p = 0.03$).

In self (-sc) competition, the families showed a similar trend in seed yield to plants grown in isolation. Although only the difference between 4A4 and 13B6 was significant ($p = 0.02$), both (spring fast growing) 8A4 and 4A4 yield more seeds than their slow growing partners. The rank order of seed yield of the families was observed to remain approximately the same as for solitary plants.

All plants in non-self (-c) competition produced marginally more seed yield than in self competition. For example, family 13B6 was observed to produce more seeds than plants grown in self competition (as well as in isolation). Similar to plants grown in isolation and self competition, 8A4 and 4A4 grew more seeds than 11B2 and 13B6. These differences however, were not statistically significant.

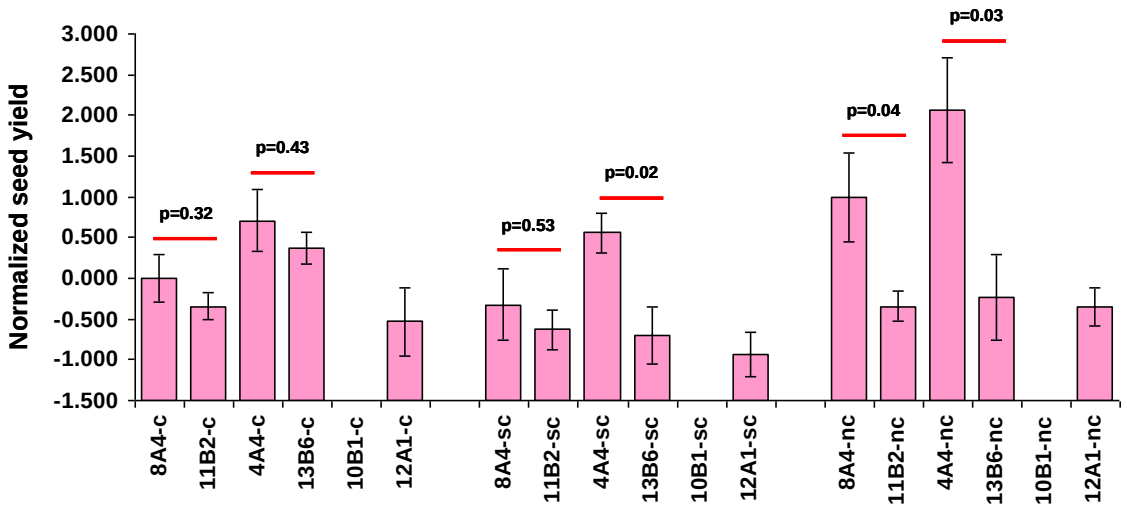

Figure 6-5 **Seed yield from solitary and competing individuals from LE in spring**. Data are presented as in Fig. 6-4. Note: 10B1 die of drought before seed matured.

Discussion

This study illustrates the possibility of predicting, to a certain degree, growth of local genotypes under competition in the field during autumn and spring. The results from the experiments also suggest that neighbouring plants could affect fitness of the genotypes as a consequence of competition, but that the outcome of competition could broadly predicted from the performance of the genotypes in isolation. In addition, the rank performance of families does not change dramatically in competition, suggesting the relationship between growth rate and fitness found in solitary plants is relevant to their performance in competition.

The significance of growth differences in competition under natural conditions

Relative growth performance at LE acted as an effective predictor of relative growth at HE, when grown both as solitary individuals and in non-sibling competition. This is supported by highly significant correlations between growth at LE and HE for solitary plants or for plants grown in competition. This suggests that elevation does not significantly influence the effects of competition; otherwise the growth correlation between elevations would be weakened.

The difference in performance of some families from previous experiments could be attributed to the influence of differences in the seasonal environment. The competition experiment was performed during an unusually warm autumn (approximately 5ºC warmer than previous average autumn seasonal temperature, Chapter 4); this may have induced the slower growing such as family 4A4 to show better relative growth performance. If family 4A4 is assumed to be adapted to warmer temperatures in spring, then its growth rate could be markedly improved in an autumn that is warmer than normal.

Support for this idea comes from the growth performance of 4A4 in the autumn of the competition experiment. Solitarily grown 4A4 was marginally (but significantly) slower than 13B6 (Fig. 6-2). This contrasts with family 4A4 being recorded as one of the fastest growing families in spring (Fig. 6-3). It is plausible therefore that the performance of 4A4 is the result of adaptation to a particular seasonal condition, with the importance of this adaptation being further intensified by the pressure of competition from non-sibling individuals.

The presence of competitors had different influences on the growth rate of some genotypes, both in autumn and spring. Growth differences between most competing (sib or non-sib) and solitary plants of the same genotype were significant (Table 6-2A, B). In autumn, most competing plants were observed to have slower growth rates than non-competing cohorts. The difference in growth rate between competing and solitary plants was greater for some genotypes at LE than others. In all cases, competing plants in spring grew faster than non-competing cohorts: this is a reversal of the situation in autumn.

A possible explanation for these different growth outcomes in different seasons is that the effect of competitors upon growth is partially dependent upon environmental conditions (Wilson 1988; Lee 1960). During the autumn months, natural resources gradually become more restricted: for example, light becomes restricted by shortening day length. Growing with a competitor could mean an additional stress that may slow growth. During spring, the faster rate of growth of competing plants could be attributed to a less stressful environment, with a longer day length and warmer temperature. Competition in spring could induce more vigorous growth, to establish dominance over local resources, than would otherwise be required in solitary growth.

Interestingly, the classic shade avoidance (SA) phenotype of lengthened petioles and altered morphology of leaf blades (considered an adaptation to obtain better light access), was not observed in competing plants during either season (personal observation, Fig. 6-1). Light signals are perceived by plants in the form of red (R) and far red (FR) light

ratio. The R:FR ratio varies very little with seasonal conditions (Holmes and Smith 1977) but is altered significantly by reflection from neighbouring vegetation (reviewed by Franklin 2008; Kebrom 2007; Smith 1997). The lack of SA observed in autumn and spring competition experiments suggest that faster growth in the presence of neighbouring competitors occurs through other chemical or physical means or a change in light quality through reflection that was not sufficient to cause SA morphology.

Growth variation with different competitors

The identity of competitors may influence growth rate in some genotypes. A few families showed significantly slower growth when competitors were siblings, than when competitors were non-sibling. Similar behaviour to this, indicating a possible response to kin recognition, has been previously observed in other plant species (Dudley and File 2007; Falik *et al*. 2003; Callaway 2002). For example, Gruntman and Novoplansky (2004) have reported self and non-self discrimination in root growth of *Buchloe dactyloides* (buffalo grass). They found that root growth was reduced when siblings were grown together, and conversely root growth increased when non-siblings were competing neighbours. These results demonstrated that *B. dactyloides* was able to respond differently to sib and non-sib neighbours, leading authors to further suggest that *B. dactyloides* may have the ability to recognize and avoid competition with kin neighbours.

The observed slower growth rate for some genotypes in competition with siblings in this study suggests the possibility that these local *A. thaliana* genotypes have some degree of kin-recognition ability. It is possible that this ability has been acquired (selected) as a consequence of kin selection pressure, potentially in conjunction with environmental factors. One mechanism for such kin recognition may involve complex chemically signaling, via root systems, as suggested by Biedryzcki *et al*. (2010), who found that root exudates were responsible for kin identification recognition in *Arabidopsis thaliana*. Similar to other studies, seedlings grown with siblings (kin) showed less root growth. A root secretion inhibitor, sodium orthovanadate (Na_3VO_4), was shown to prevent seedlings

from recognizing ‘stranger’ (non-kin) individuals: this resulted in increased lateral root growth when seedlings were grown with non-kin competitors.

However, it is also possible that the reduced growth rate in sib competition of local *A. thaliana* is simply due to more intense competition. Siblings may compete more strongly with each other than with non-siblings - sibling individuals will share adaptations to particular environmental niches, including sharing growth attributes and associated requirements. This entails that these kin are in direct competition, yet sharing the same (adapted) ability to access these environmental resources. Rather than competition not being present (as with kin recognition) amongst siblings, competition would be viewed as having an *equal effect* upon siblings due to selection pressures applying equally across them. Consequently, these kin individuals would all be expected to display equally reduced growth, reflecting the fact that none have a genetic advantage over their sibling rivals.

Besides, the observed greater growth differences when plants are growing with non-siblings could be due to the effects of niche partitioning. Niche partitioning is commonly considered to occur individuals that live in close proximity may access limiting resources in a different manner (i.e. different rooting depth), therefore reducing or avoiding competition (reviewed by Silvertown 2004). Similarly, non-sibling individuals may have slightly differing resource requirements, as the outcome of adaptations from inhabiting different environmental niches (i.e. seasonally adapted). This disparity could act to reduce the level of effective competitive pressure.

Fitness in competition

The relative fitness of most plants was observed to correspond (to a degree) with their seasonal preference - seasonally adapted individuals are fitter during their preferred season. This is supported by the higher seed yield produced by seasonal fast growing families in autumn and spring, i.e. non-sibling paired competition, particularly at LE site.

More importantly, most of these seed yield differences between fast and slow growing families were significant. Several insignificant differences in autumn yield at HE was most probably due to severe wet weather conditions at harvesting.

Plants are known to modify their allocation of growth resources, in order to alter their architectures (i.e. taller plant height and petiole elongation for better light source) when in competition (Tremmel and Bazzaz 1995). Studies have shown some agricultural crops such as peas (*Pisum sativum*) (O'Brien *et al*. 2005) and Kenya beans (*Phaseolus varigaris*) (Maina *et al*. 2002) compromised seed yield production as a result of growing significantly more roots in response to competition. Similar to the outcome of peas and soybean plants studies, it was observed here that the seed yield of local *Arabidopsis thaliana* grown in competition decreased.

In particular, growth performances of spring competing pairs were observed to be greater than solitary plants, but their seed yield were less than those in isolation. A phenomenon describe by Gersani *et al*. (2001) in root study of soybean plants would seen appropriate to explain the observation here. Gersani *et al*. (2001) investigated intra- and interplant competition on root proliferation by growing soy bean plants in competition and isolation. Split root seedlings were created (the distal root tip was removed after two days of sowing to induce growth of two near identical roots), and grown both in isolation (each half of the root within an individual pot; referred as owners) and as pairs sharing two pots (each member of the pair straddling the two pots), such that one half of its roots were in each pot (referred as sharing individuals). Normal seedlings were also grown, again with individuals in isolation (owners) or in pairs sharing the same pot (sharing individuals). In all cases, total plant biomass did not differ significantly. However, it was found that root mass was significantly increased for individuals in direct (interplant) competition (sharing individuals; both split roots straddling in two pots and two normal plants sharing one compartment), whereas plants in isolation had higher seed yield (more seed pods, more seeds per pod and greater seed mass). One of the suggestions from this work is that over grown (significantly different in shoot and root ratio) organs (roots in

this case) incurred a greater resource cost, but without a relative increase in competitiveness.

Although the tendency for seed yield reduction due to more root growth may or may not applicable to *A. thaliana*, the effects of competition on seed yield in local *A. thaliana* suggests that competition has a negative effect on reproductive growth. Alternatively, this outcome could simply be the result of increased ground resource depletion - as two individuals accessed the same level of resource as a single plant grown in isolation.

It is obvious that further experimental data will be required to confirm these observations, through providing data from a variety of seasonal and environmental conditions. As natural environment studies are always likely to be subject to regular and unpredictable climate fluctuations, a wide cross-section of varying conditions is required to more accurately assess and determine the influence of each environmental factor. This can only be achieved through long term studies that follow the natural variation of such factors over time.

Chapter 7 Effects of abiotic factors on seasonal growth

Introduction

To understand how plants have adapted - and continue to adapt - to their environments, numerous studies have investigated the involvement of various abiotic factors, such as photoperiod, temperature and irradiance, in different developmental stages, as early as germination, both under controlled conditions and in a wide range of natural environments (Huang *et al*. 2010; Pieters 1999; McWilliam and Naylor 1967). Plants in cold environments, such as arctic tundra and high alpine, for example, are adapted to combinations of cold temperature, drought, and high irradiation. Plants that do not show growth and development adapted to those specific environmental qualities – such as desert plants – would struggle to compete and even survive in such environments.

Photoperiod is defined as the amount of light and darkness in a 24 hour daily cycle. Three principle photoperiod responses in plants have been described. Short-Day plants respond to a shorter photoperiod than the critical day length (CDL; the point where photoperiod switches from non-inductive to inductive); in contrast, Long-Day plants respond to a photoperiod exceeding the CDL. Finally, Day-Neutral plants do not have a response to photoperiod (reviewed by Jackson 2009).

Photoperiod may sometimes appear to be less important than other environmental variables, such as temperature and water availability. For example, in arid habitats - such as desert - response to water availability, which may be seasonal but unpredictable within a season, is arguably more critical than response to changing day length. Similarly, in equatorial environments the day length is relatively constant (12 hours daylight) and thus there is more scope for plants to adapt to other, more variable, factors such as temperature or precipitation. Nonetheless, photoperiodic control of flowering is observed in many species and might be adaptive because of the direct relationship between flowering and reproductive success (Hancock 2012; reviewed by Jackson 2009).

Adaptive differences in response to photoperiod have been reported for many plant species. In a classical study, McMillan (1973) collected samples of the weed cocklebur, *Xanthium strumarium*, from across the Chihuahuan Desert and Sonoran Desert at the border between the USA and Mexico. These were then grown under controlled conditions and in a common garden located in Central Texas, USA. *X. strumarium* populations were found to have different critical night lengths, between 9.5 (short night, longer day) and 10.5 hours (long night, shorter day) for flowering. In the common garden experiment, McMillan found that Chihuahuan populations from the westerly site of the desert flowered in short nights (during early spring) whereas the easterly populations delayed flowering until the longer nights in late summer. This observation correlated with the differences in the length of growing seasons (the easterly site was less likely to experience late frosts), as well as predictability of precipitation (the easterly site was wetter through the summer). It was suggested that each population might be adapted to different photoperiod cues, to maximize utilization of local environmental conditions.

Temperature is one of the most studied environmental variables in adaptation; many organisms (i.e. cyanobacteria and glacier ice worms) are adapted to extreme temperatures. In plants, temperature is one of the most important factors affecting growth and development. For example, photosynthesis is strongly influenced by temperature. Plants exposed to temperatures below or above a range of 10ºC to 35ºC can experience damage to sensitive components, such as enzymes, membranes and pigment-protein complexes, resulting in photo-oxidative damage (Berry and Björkman 1980). In the field, temperatures can vary greatly across different seasons, elevations and latitudes and species from different habitats show adaptations to local temperatures, enabling efficient functioning of photosynthesis under the temperature regimes of their native habitats (Berry and Björkman 1980).

Plants - such as those in temperate regions - grow within a range of temperatures. Well adapted plants are believed to closely match their growth rate to temperature during a growth season. Criddle *et al*. (2005) tested adaptation of *Eucalyptus camadulensis* and

Artemisia tridentate (sagebrush) to temperature. They developed a thermodynamic model of plant growth rate, based upon the metabolic rate (measured as the CO_2 output) and the efficiency of carbon to energy conversion. Their models supported the idea that environmental temperature can be mechanistically linked to the biochemical reactions of plant growth.

Plants can also be physiologically adapted to a changing environment – the same genotype can display different phenotypic characters in response to environmental changes (plasticity) and species can differ in their ability to adapt in this way (reviewed by Lütz 2010). Griffith *et al*. (2007) for example, reported growth and development of *Thellungiella salsuginea* - a close relative of *A. thaliana* that grows as either biennial or annual in subarctic Canada. They showed the amazing ability of *T. salsuginea* to thrive under freezing temperature in cold controlled chamber experiments. They also examined cold regulated (*COR*) genes and found that *COR* and *CBF* genes (which encode a family of cold-induced transcription factors, the C-repeat binding factors, that regulate transcriptional activity of *COR*) were strongly up-regulated in response to cold. In particular, *COR47* expression was highest in response and *COR15a* transcription levels accumulation were greatest after just 24 hours of freezing treatment. This might explain the ability of *T. salsuginea* to adapt to extreme freezing (also described as 'supercooling') conditions.

Irradiance has various implications for living organisms – and is particularly importance when it provides energy for growth. Irradiance adaptations can be seen in prokaryotes such as the photosynthetic marine green sulfur bacterium, *Chlorobium BS-1*, which is adapted to the extreme low-light conditions of the Black Sea (0.001 µmol $m^{-2}s^{-1}$ during winter), yet still relies upon light for survival (Marschall *et al*. 2010). Adaptation of plants to irradiance, however, is often associated with photoperiod and temperature (Diaz *et al*. 2007, Wentworth *et al*. 2006, Pieters *et al*. 1999).

Others, including Külheim *et al*. (2002) have examined the regulation of photosynthesis in different irradiance levels. They used the mutants *npq1* and *npq4*, which lack proteins

essential for feedback de-excitation against photo-oxidative stress under high irradiance condition. *npq1* lacks the enzyme violaxanthin de-epoxidase (VDE) that converts violaxanthin (a carotenoid species) to zeaxanthin, and *npq4*, lacks PsbS that undergoes a confirmation change when irradiance pressure rises. The feedback de-excitation regulates and balances harvesting of light with metabolic energy consumption in plants, by switching the photosynthetic antennae so that they dissipate light energy as heat, rather than directing it towards metabolism. Külheim *et al*. demonstrated that loss of feedback de-excitation strongly reduced plants fitness under natural and controlled conditions. They suggested that it offers an adaptive advantage by providing short-term photosynthetic regulation than protection against high irradiance.

This chapter is focused on the growth performance of selected local genotypes under various controlled environments. These experiments were designed to identify the environmental variables that differentially affected the growth of local accessions in seasonal field studies in spring and autumn.

Results

These investigations were performed to test the effects of abiotic variables upon growth rate. Families were selected based on their previous seasonal performance to represent a range of different responses. Because these families had been found to respond differently to spring and autumn environments, they were grown in different photoperiods, temperatures and light intensities that served to simulate the average conditions plants had experienced in the field. Development was monitored regularly and rosettes were photographed twice a week for growth rate estimation. Relative growth rates were calculated for the controlled environment studies and compared to growth rates in the field.

Growth in conditions simulating seasonal climate

The families examined in this chapter are summarized in Table 7-1; three to eight members per families were used in each experimental condition. The conditions varied for temperature (from 20°C to 5°C), photoperiod 16 hour light (LD) or 8 hours (SD), high irradiance (HL, 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or low light (LL, 135 $\mu mol \cdot m^{-2} \cdot s^{-1}$) intensities was also investigated in a controlled environment greenhouse (GroDome) at 20ºC and LD conditions. The combinations are shown in Table 7-1.

Facilities	**Growth room**				**Growth cabinet**						**GroDome**	
Temp (ºC)	**20**		**16**		**16**		**10**		**5**		**20HL**	**20LL**
Photoperiod	**LD**	**SD**	**LD**	**-**	**LD**	**SD**	**LD**	**SD**	**LD**	**SD**	**LD**	**LD**
1B5	√	√	√	-	√	√	√	√	√	√	√	√
1D1	-	-	-	-	√	√	√	√	√	√	√	√
2C2	√	√	√	-	√	√	√	√	√	√	√	√
2E1	√	√	√	-	√	√	√	√	√	√	√	√
2E5	-	-	-	-	√	√	√	√	√	√	√	√
4A4	√	√	√	-	√	√	√	√	√	√	√	√
5A3	√	√	√	-	√	√	√	√	√	√	√	√
6A3	√	-	√	-	√	√	√	√	√	√	√	√
7B5	-	-	-	-	√	-	-	-	√	-	√	√
8A3	-	-	-	-	√	√	√	√	√	√	√	√
8A4	-	-	-	-	√	√	√	√	√	√	√	√
9A2	-	-	-	-	√	√	√	√	√	√	√	√
10A1	√	√	√	-	√	√	√	√	√	√	√	√
10B1	-	-	-	-	√	√	√	√	√	√	√	√
11A2	√	√	√	-	√	√	√	√	√	√	√	√
11A5	-	-	-	-	√	√	√	√	√	√	√	√
11B2	-	-	-	-	√	√	√	√	√	√	√	√
12A1	√	√	√	-	√	√	√	√	√	√	√	√
13A2	-	-	-	-	√	√	√	√	√	√	√	√
13B6	√	√	√	-	√	√	√	√	√	√	√	√

Table 7-1 **Summary of various controlled conditions**. Selected genotypes were examined under various conditions. Between three and eight plants per family were used in each experimental condition (see 2-5-2 for further details of population sizes). Keys: LD = long day, SD = short day, HL = high light intensity, LL = low light intensity, "√" = tested, "-" = not tested.

Effects of photoperiod and temperature on growth

Plants at 20ºC were observed to grow faster in LD than SD (Table 7-2). The growth rates of the different genotypes, however, were well correlated between photoperiods ($r = 0.70$, $p = 0.03$). The significant correlation indicates that fast growing families (i.e. 10A1) in LD tend to remain fast in SD, and *vice versa* for slow growing families (i.e. 2E1) (Fig. 7-1). However, plants at 16ºC LD grew more slowly (Table 7-2), and growth at 16ºC was not correlated with growth at 20ºC ($r = 0.04$, $p = 0.92$).

The fall in temperature (from 20ºC to 16ºC) had little effect on most genotypes but affected 2E1, 4A4 and 11A2 in different ways. Both 2E1 and particularly 4A4 grew significantly better, relative to other genotypes, under the cooler conditions ($p = 0.06$ and 0.004, respectively in Student's t-tests). However, family 11A2 showed the opposite response. This suggests that genotypes might be more adapted to differences in temperature than photoperiod and that photoperiod had little effect on relative growth performance, at least under warm conditions.

Families were further compared in LD and SD conditions at additional lower temperatures (Table 7-3). In general, correlations between growth in LD and SD were significant and similar at colder temperatures ($r = 0.46$, $p = 0.05$ at 5°C and $r = 0.50$, $p = 0.03$ at 10°C), but not significant at 16ºC ($r = 0.07$, $p = 0.76$).

Correlations between growth at 5ºC and 10ºC were significant in both LD ($r = 0.56$, $p = 0.01$) and SD ($r = 0.45$, $p = 0.05$). In contrast, no correlation could be detected between relative performance at 5ºC and 16ºC under either photoperiod (Table 7-3). Additionally, Growth at 10ºC was significantly correlated to growth at 16ºC under SD conditions ($r = 0.81$, $p < 0.0001$), but not under LD. Growth rates in all the controlled conditions were highly heritable. For example, H^2 values for 16ºC LD and 16ºC SD are 0.87 and 0.75, respectively. These results suggest that temperatures and photoperiods have substantial influences on the relative growth performances of local genotypes; the correlations have not been lost because of non-genetic variance. Otherwise, it would be expected that all

growth regressions between various temperatures and photoperiod would be significantly strong.

	Population mean area (cm^2)	± *SE*
16ºC LD	21.09	0.84
20ºC LD	40.75	1.34
20ºC SD	11.04	0.31

Table 7-2 **Population mean rosette area (cm^2) in different controlled conditions**. The rosette areas were measured at 32 dsg.

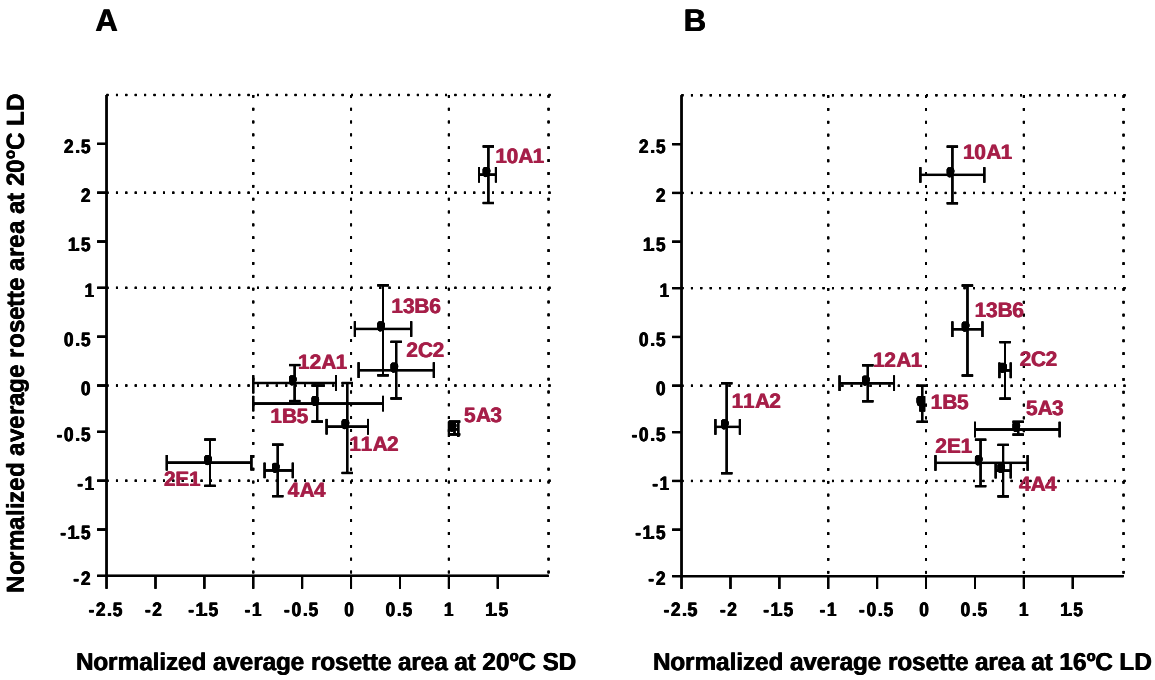

Figure 7-1 **Examples of relative growth rate in different photoperiods and temperatures**. Normalized rosette areas at 20ºC LD and SD are shown in (**A**) ± SEM. Values are well correlated (r = 0.70, p = 0.03). Growth at 16ºC and 20ºC in LD is compared in (**B**). In this case there is no significant correlation (r = 0.04, p = 0.92). The growth rate H^2 estimated for 20ºC LD and 16ºC LD are 0.74 and 0.79, respectively.

	r	p
5ºC LD / SD	0.46	0.05
10ºC LD / SD	0.50	0.03
16ºC LD / SD	0.07	0.76
5ºC / 10ºC LD	0.56	0.01
5ºC / 10ºC SD	0.45	0.05
5ºC / 16ºC LD	0.13	0.57
5ºC / 16ºC SD	0.38	0.13
10ºC / 16ºC LD	0.23	0.33
10ºC / 16ºC SD	0.81	<0.0001

Table 7-3 **Regression summary of growth rate in different controlled conditions**. Average rosette areas were used as growth rate estimates.

Effects of light intensity on growth

Plants grown under high light intensity (HL, 900 µmol·m^{-2}·s^{-1}) and low light intensity (LL, 135 µmol·m^{-2}·s^{-1}) were observed to grow at similar rates up to 17 dsg (Table 7-4), bur plants in HL were significantly larger than in LL after 24 dsg. However, the rosette areas at 17 and 24 dsg within each treatment, were significantly and strongly correlated – HL; $r = 0.91$ $p < 0.0001$ and LL; $r = 0.90$, $p < 0.0001$ (Fig. 7-2). In addition, growth in HL and LL were significantly and strongly correlated ($r = 0.84$, $p < 0.001$); indicating that fast growing families at HL remain fast in LL, and vice versa for slow growing families (Fig. 7-3). This suggests that light intensities may not be a key factor in changing the relative ranking of growth in these local genotypes.

	Population mean area (cm^2)			
	17 dsg	**± *SE***	**24 dsg**	**± *SE***
HL	1.1	0.025	8.5	0.165
LL	1.0	0.027	6.0	0.125

Table7-4 P**opulation mean rosette area (cm^2) in different light intensity conditions**. The rosette areas were measured at 17 and 24 dsg.

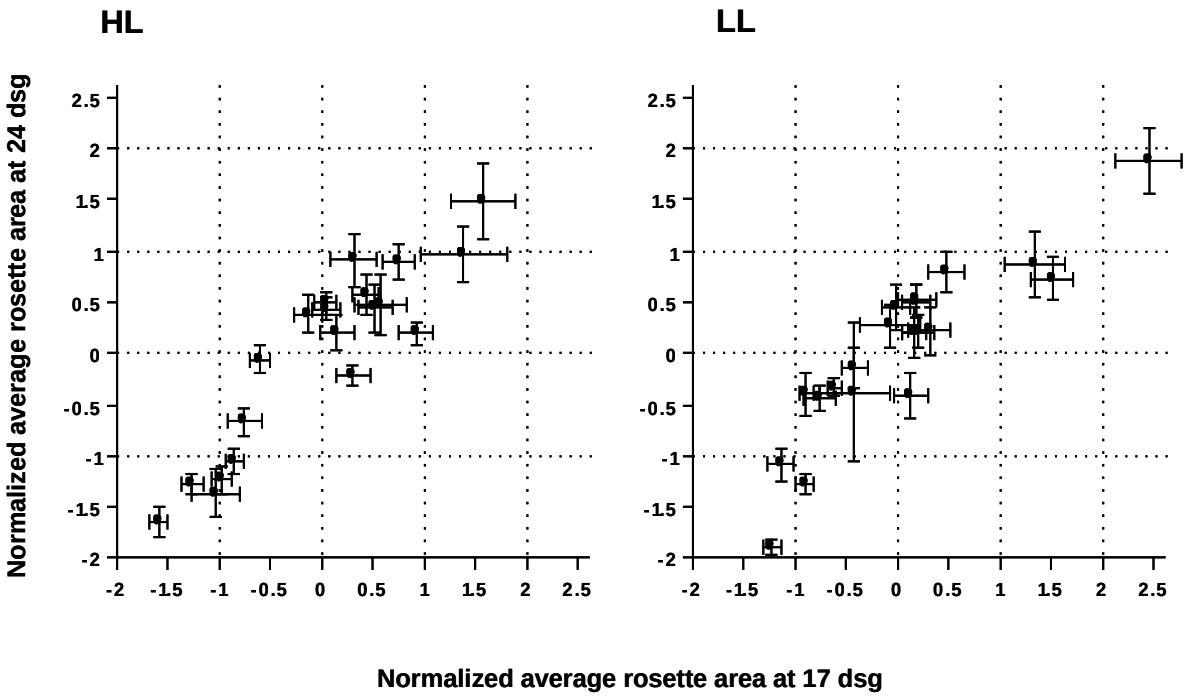

Figure 7-2 **Regression of normalized rosette areas at 17 and 24 dsg in different light intensities.** The relationships are shown under high light (**HL**, 900 µmol·m^{-2}·s^{-1}) and low light conditions (**LL**, 135 µmol·m^{-2}·s^{-1}). Mean values for each family are used ± SE. Rosette areas are highly correlated at 17 and 24 dsg in both HL and LL ($r = 0.91$, $p < 0.0001$ and $r = 0.90$, $p < 0.0001$, respectively).

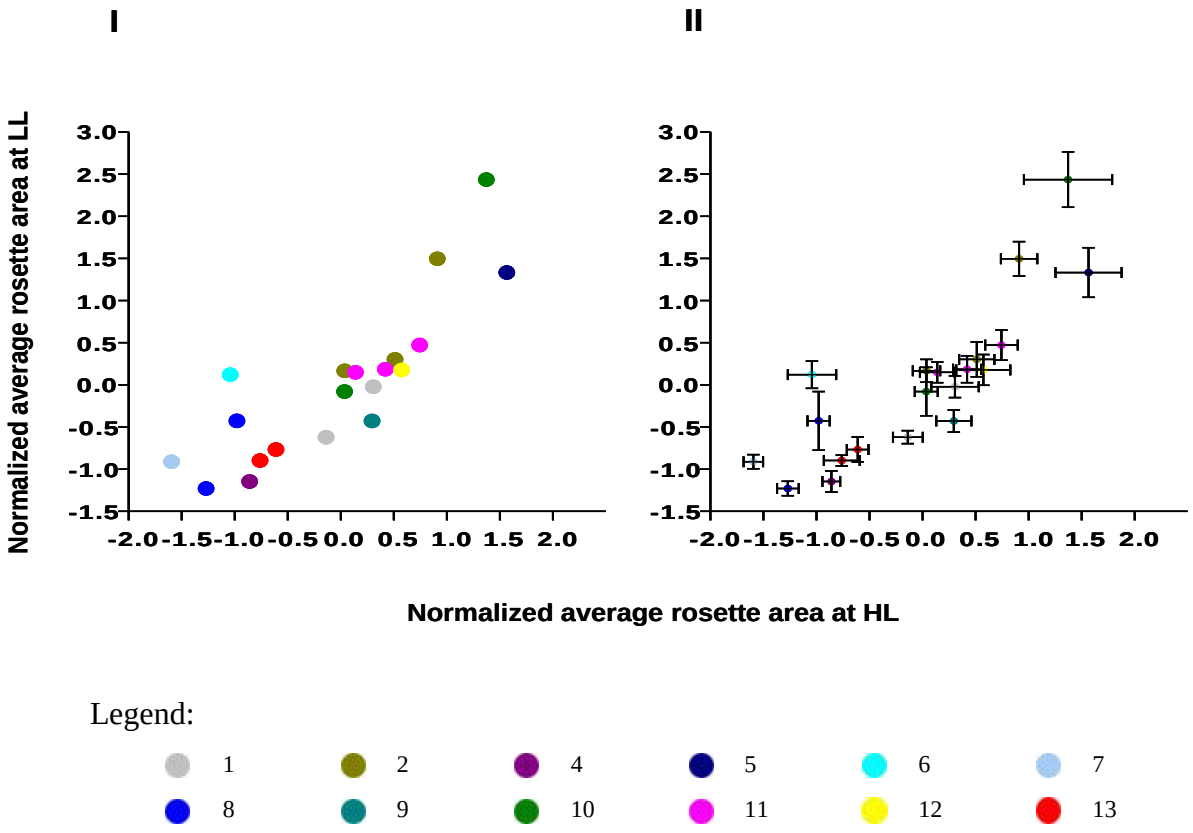

Figure 7-3 **The relationship between growth in HL and LL**. Normalized average rosette areas at 17 dsg are shown. Growth rates in HL are strongly correlated with those in LL ($r = 0.84$ $p < 0.0001$). Plant families are colour coded based upon the sampling locations (1 to 13) for the local accessions (**I**) with ± *SE* (**II**).

Ranking summary for growth under controlled environments

The families were ranked according to their growth rate within each experiment, as summarized in Figure 7-4. Some families showed similar relative growth performance in all conditions (various temperatures, photoperiods and irradiance), whereas some grew better under some conditions. In all controlled experiments, families 5A3 and 10B1 were among the fastest growing families; with growth that was the least affected by environment. In contrast, families 9A2 and 12A1 grew relatively poorly in all conditions when the temperature was below 16ºC.

The rankings also indicate that some families, such as 2C2, 2E1 and 2E5, perform better in warmer temperatures (above 10ºC) regardless of photoperiod (Fig. 7-4) and that they improved their relative growth performance in line with increasing temperatures. For instance, 2C2 performed better at temperatures above 5°C while 2E1 performed better at 16ºC or above. In addition, some families performed better under a specific photoperiod. For example, family 1D1 always grew better in LD than SD, regardless of temperature (5ºC to 16ºC). Family 11B2 showed the opposite response, as it grew faster in SD than LD.

However, growth rankings of some families were strongly affected by a particular combination of photoperiod and temperature, suggesting a more complex interaction between these variables. Families such as 1B5, 4A4, 8A4, and 13A2, showed opposite responses to photoperiod that were conditional on the temperature. As a general trend family 1B5 grew better at warm temperature (16ºC) and 4A4 grew better at cold temperature (5ºC) regardless of photoperiod. However, at the intermediate temperature of 10ºC, photoperiod became a key factor in influencing growth: 1B5 grew faster in LD and 4A4 in SD (Fig. 7-4).

An influence of photoperiod was also observed in families 8A4 and 13A2. However, in this case photoperiod was crucial at the opposite ends of the temperature range. The growth ranking summary indicated that family 8A4 grew slowly at temperatures above 10ºC regardless of photoperiod; conversely, 13A2 grew slowly at temperatures *below* 10ºC, regardless whether they were grown under LD or SD. However, at 5°C, 8A4 grew faster in SD than LD, and 13A2 grew faster in LD than SD at 16°C (Fig 7-4).

Light intensity did not affect the growth rankings of most families. Only two showed significant differences. Family 9A2 performed significantly better in HL, and 6A3 in LL ($p = 0.03$ and $p < 0.01$ respectively in Student's t-tests).

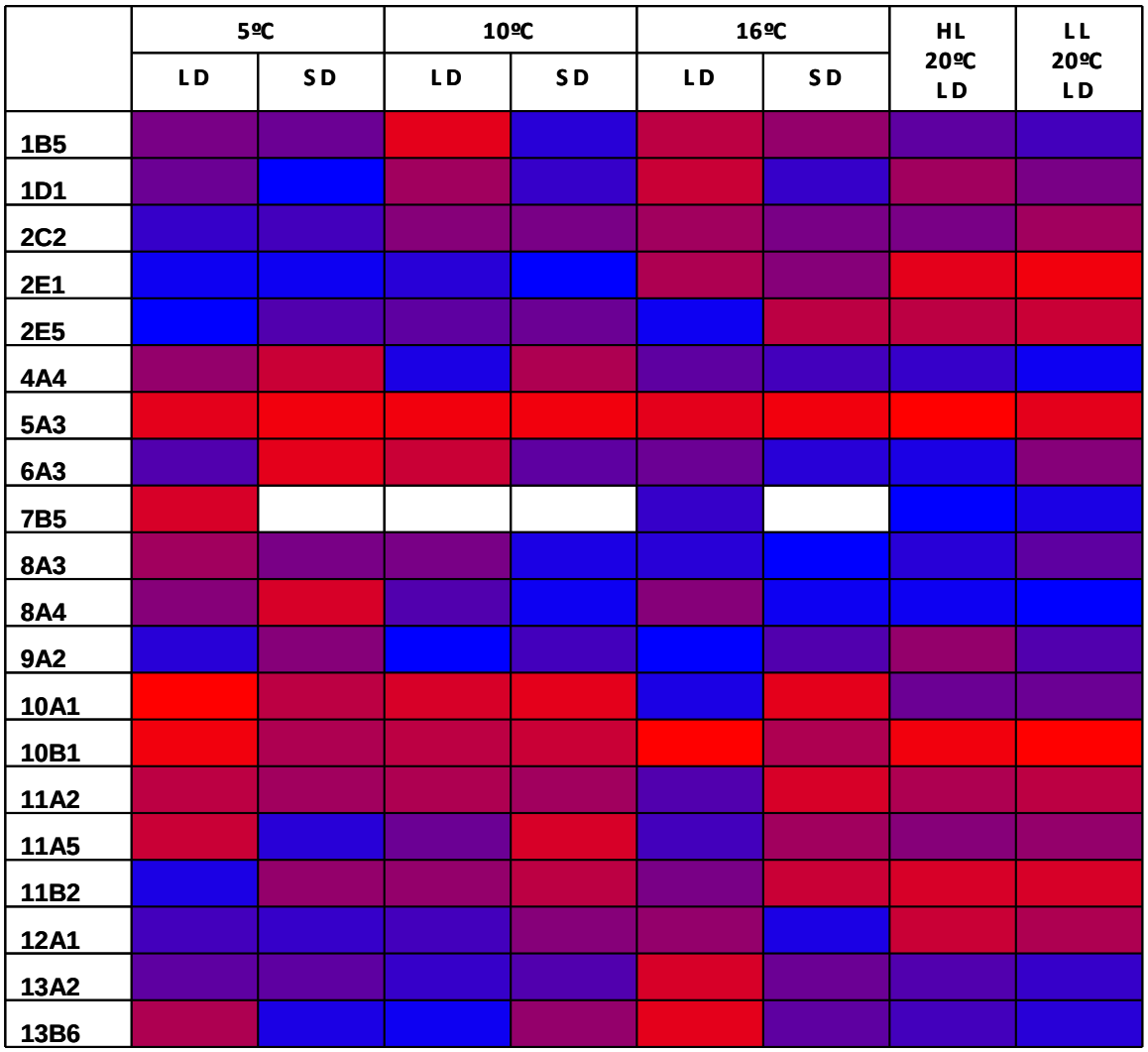

Figure 7-4 **Growth ranking of 20 selected families in response to temperature, photoperiod and light intensity**. The families within each experiment were assigned a colour to represent their position in the ranking of growth rate based on average rosette areas. Red represents the fastest growing family in the experiment and blue the slowest. Family 7B5 was not included in some of the controlled experiments due to poor germination.

Relative growth rate, RGR, in natural and controlled environments

RGR was calculated using rosette area estimations made one week apart. The earliest RGR values in the seasonal experiments (natural environments) were calculated from measurements at 18 dsg and 25 dsg. In autumn, measurements were taken until December and in spring until May, when plants began to bolt. Measurements in GH and controlled experiments began when rosettes had reached a sufficient size to allow accurate estimates of their areas and continued until the first bolting in the population was observed.

Figure 7-5A shows plots of RGR over time in autumn and spring at LE. In autumn (blue) RGR declined sharply over approximately the first 5 days of measurement, and then increased to peak at approximately 38 days (i.e. 0.200 $cm \bullet cm^{-1} \bullet days^{-1}$). RGR subsequently declined to around 0.070 $cm \bullet cm^{-1} \bullet day^{-1}$, staying at this rate for approximately 10 days before constantly declining until the end of the experiment. In contrast, RGR of spring plants (red) showed a less dramatic overall decline in RGR during the course of the experiment, but also exhibited a transient increase in RGR at around 39 days after germination.

Similar variation in RGR was observed in GH conditions, except with a reduced difference between autumn and spring grown plants (Fig. 7-5B). The RGR fluctuations at GH were generally lower and most plants in autumn did not show a transient peak in RGR at around 38 days.

This patterns of RGR with time could be intrinsic (i.e. always occur during development of the plants) or could reflect a responses to the environment. For example, the latter would be consistent with falling growth rate with decreasing temperature, day length and light intensity in autumn. One way to distinguish the contribution of internal and external factors is to grow plants in a constant environment. Therefore RGR was calculated for plants grown in controlled growth cabinet (Fig 7-6). At 10 or 16ºC plants continued to show a decrease in RGR with time, suggesting that it may be an intrinsic property.

However, less fluctuation in RGR was seen in the constant environments. This included loss of the RGR peak at around 38 dsg, suggesting that this peak occurred in response to an environmental changes in seasonal field experiments. The increase of RGR at this point in both autumn and spring could result from an increase in temperature – there was a short warm spell in autumn around 28 to 40 dsg, whereas in spring, temperature usually started to warm up around the same time period.

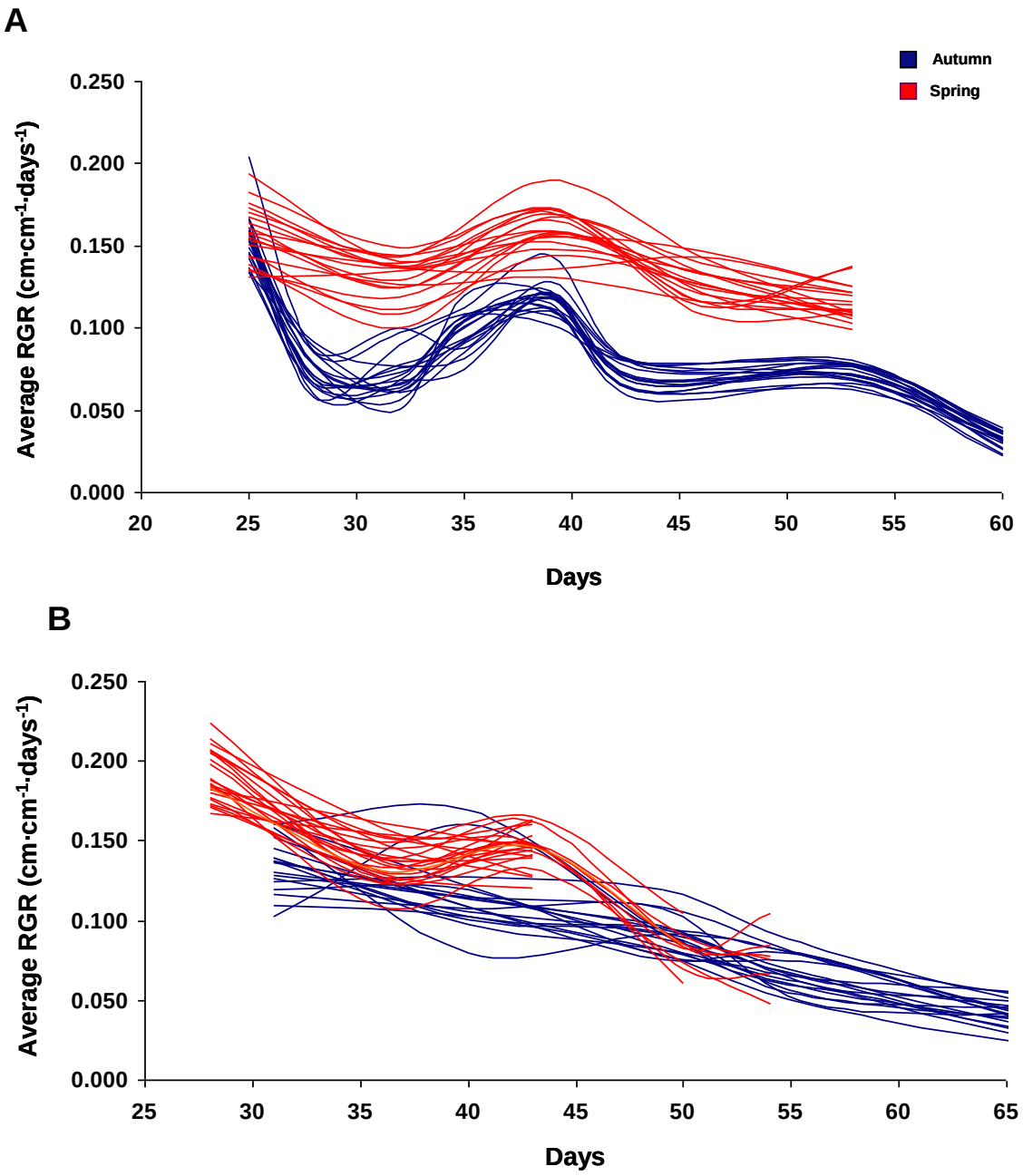

Figure 7-5 **Variation in relative growth rate over time in the field and greenhouse**. RGR was estimated from successive rosette images at LE (**A**) and GH (**B**). Each line in the represents a family's average RGR. Autumn plants had lower RGR (blue) than spring plants (red). Seasonal RGR fluctuates more in the field than in greenhouse.

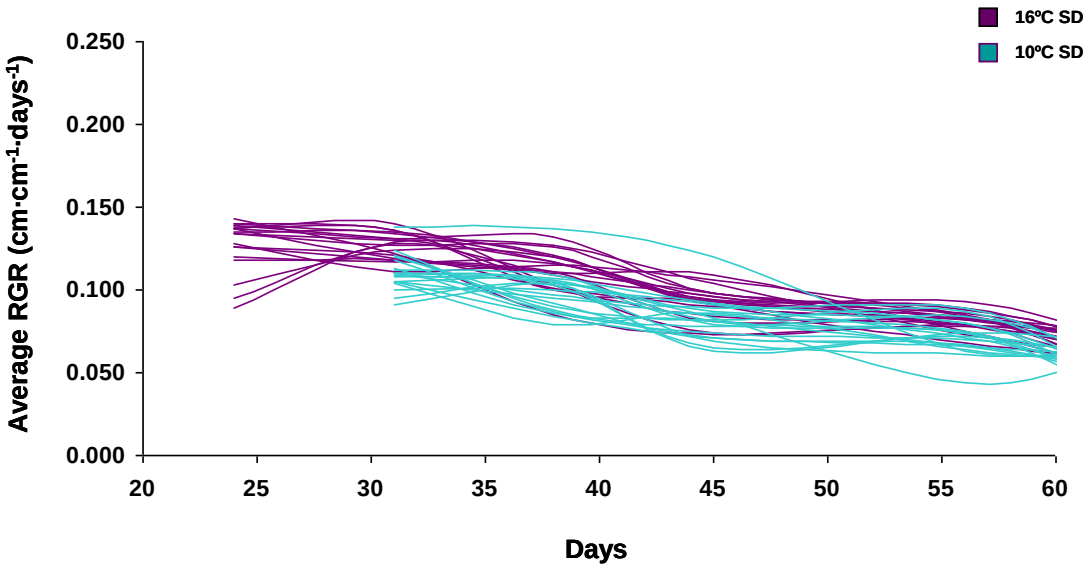

Figure 7-6 **Relative growth rate in controlled conditions**. RGR was estimated as in Fig. 7-5, for plants grown in a controlled environment (10 or 16ºC, at least 100 days until most plants bolted). RGR fluctuates less under controlled conditions compared to natural seasonal conditions shown in Fig. 7-5. Keys: SD = short day.

Heritability, H^2, estimated from fluctuating RGR

Broad-sense heritability, H^2, was estimated for RGR values calculated at different time points in field and controlled environments (Table 7-5). The estimate of H^2 changes with time, as (being non-constant) environmental factors have varying influence upon the phenotypic trait; in this case the growth rate. This highlights the importance of the RGR sampling time, as the H^2 estimate indicates the degree to which that particular RGR can be considered a consequence of genetics rather than environment.

Most H^2 values (within each experiment) were found to be low, except a few under LE autumn and GH spring conditions. The initial H^2 estimates for LE autumn was also observed to dip at around 32 dsg, corresponding to the transient increase in RGR (shown in Fig.7-5). In summary, local accessions show genetically determined differences in RGR under both field and controlled conditions. RGR H^2 was also observed influenced differently by the environment in early growth under different conditions.

LE autumn	***25 dsg***	***32 dsg***	***39 dsg***	***43 dsg***	***53 dsg***	***60 dsg***
H^2	0.81	0.33	0.62	0.19	0.89	0.65
LE spring	***25 dsg***	***32 dsg***	***39 dsg***	***46 dsg***	***53 dsg***	
H^2	0.55	0.37	0.26	0.22	0.39	
GH autumn		***31 dsg***	***40 dsg***	***45 dsg***	***53 dsg***	***63 dsg***
H^2		0.35	0.40	-	0.37	0.41
GH spring	***25 dsg***	***31 dsg***	***38 dsg***	***45 dsg***	***52 dsg***	
H^2	0.39	0.56	0.25	0.72	0.68	
10ºC SD		***31 dsg***	***38 dsg***	***45 dsg***	***56 dsg***	***63 dsg***
H^2		0.22	0.38	0.47	0.41	0.15
16ºC SD	***24 dsg***	***31 dsg***	***38 dsg***	***45 dsg***	***56 dsg***	***63 dsg***
H^2	0.30	0.19	0.36	0.27	-	-

Table 7-5 **Summary of heritability of RGR under natural and controlled conditions**. The heritability values (i.e. for 24 dsg) were calculated from the increase in rosette area in the previous week. The same families were involved in all experiments. The estimate of H^2 changes with time, as well as depending on experimental conditions. Keys: "-", RGR differences were insignificant.

Discussion

This study demonstrates the influences of abiotic variables upon growth of local genotypes. The growth rankings suggest that some genotypes' growth rates can be influenced by a combination of multiple environmental factors. In addition, the actual growth (in functional terms) of plant in seasonal and controlled experiments was illustrated by RGR.

The significant influences of photoperiod and temperature on growth

Most local genotypes generally grew faster under LD, warm temperature and HL conditions, with almost double the growth rate under SD, colder temperature and LL conditions. Similar observations have been reported in other plant species, such as poplar (Pieters *et al*. 1999).

The effects of different environment variables upon growth varied among genotypes. Some genotypes' growth rankings were altered under specific photoperiod or temperature conditions. Examples of photoperiod-dependent performance were demonstrated by families 1D1 and 11B2; these consistently grew faster under either LD or SD conditions independent of temperature. Hypothetically, if 11B2 is adapted to SD such as experienced during autumn/ winter seasons, this would explain the faster growth in autumn than spring (as recorded during previous seasonal experiments, Chapter 5).

Further potential evidence of adaptation to specific seasonal variable (i.e. temperature) was demonstrated by family 2E1, which consistently ranked amongst the fastest growing genotypes under warm temperature (16ºC and 20ºC). Some families, like 13A2 and 13B6, were observed to grow faster under a specific combination of temperature and photoperiod; in this case, 16ºC LD. Out of this range of photo-thermal combination, both 13A2 and 13B6 were not seen to grow particularly fast compared to other genotypes (Fig. 7-4). Alteration of the rankings (from slower to faster growth, and vice versa) provides supporting evidence that some local genotypes may be adapted to specific environmental variable(s), explaining their contrasting growth performance under different conditions.

In some cases, however, the growth of some genotypes was observed to be influenced not purely by photoperiod or temperature, but by an interaction between both variables. A possible explanation is there may be varying priorities given to environmental signals perceived by plants, where the (effect of the) interaction of influences may change depending on the strength of a primary, predominantly influencing, variable. These genotypes, such as 10A1 and 11A2, were observed to be fast growing under all condition except at 16ºC LD. For example, if both 10A1 and 11A2 were biennial (adapted to cold SD conditions) and their growth is predominantly influenced by temperature, then they would be expected to grow better under cold conditions, as indicated in Figure 7-4. At 16ºC - a temperature associated with warm autumnal conditions (i.e. similar to the temperature recorded in autumn 2009; Chapter 4) - then it is possible that the primary temperature influence is insufficient for the normal cold-weather adaptation response (i.e. it is neither cold enough to indicate winter nor warm enough to signify summer). An

alternative seasonal element, such as photoperiod, acting in combination with temperature could dominate and act as a signal for fast growing in this case.

Another example of growth affected by the interaction of photo-thermal interaction was observed in 4A4 (Fig. 7-4). The growth speed in various controlled conditions suggested that 4A4 is adapted to be biennial - with faster growth under cold SD conditions, but growing at a slower rate in warm LD.

Interactions involving multiple variables with one over-riding the other, i.e. temperature response, have been recorded in perennial species. *Artemisia tridentata* is cold desert shrub that grows in Utah, USA. It was observed that *A. tridentata* grew better at lower temperatures associated with early spring, but in midsummer water stress (due to seasonal drought) became the dominating factor in photosynthetic performance (DePuit and Caldwell 1973).

The temperature dependent responses to photoperiod observed between 10A1 and 4A4 (i.e. 10A1 grew faster at 16ºC LD than SD, whereas 4A4 grew faster at 10ºC SD than LD) may simply reflect differences in optimum temperature and these differences might be adaptive; 4A4 came from around 60 m asl and 10A1 from around 150 m asl, which was on average 2°C colder (Chapter 4). Local adaptation to temperature could also explain differences in temperature sensitivity of the genotypes. For example, plant populations from higher elevations might be more sensitive to high temperature, due to adaptation to colder temperature - and vice versa for low elevation populations.

Importantly, this also indicates that adapted growth performance of local *A. thaliana* populations may involve a complicated signaling mechanism, involving the integration of multiple environmental signals. A perfect adaptation would not be possible in natural environments, because seasonal weather conditions vary from year to year.

Winter or summer annual?

In terms of the life history of populations examined in this thesis, the growth rankings suggest that local genotypes could potentially be split into three groups: 1) winter annuals, which grow better in cold SD conditions; 2) summer annuals, which germinate and flower within a season and grow better in warm LD conditions and 3) generalists, which grow well all year round. This can be inferred using growth results of multiple sets of seasonal cohorts, which show groupings of similar growth performance in natural conditions; some genotypes grew faster in autumn (cold SD), some grew faster in spring (warm LD), some grew fast in both seasons (Fig. 5-1-4, Chapter 5).

Families which appeared to grow slowly under all field conditions appeared anomalous. For example, 2E1 and 12A1 grew slowly in all seasonal experiments, and in all controlled experiments at 16ºC and lower. However, they performed better at 20ºC (in HL and LL controlled experiments), suggesting that these families may be annuals that are adapted to warm summer temperatures. In seasonal experiments (from 2007 to 2010), the average seasonal min and max temperatures are approximately 6ºC and 13ºC for autumn, 0ºC and 7ºC for winter, and 4ºC and 12ºC for spring. Hence, these families had not been tested under warmer natural conditions, such as temperatures around 20ºC found in summer and it remains possible that they would out-perform other families under these conditions.

Families that grew well as both winter and summer annuals (generalist) were exemplified by 5A3 and 10B1. They performed well in all field and controlled conditions. Weedy species such as *A. thaliana* are likely to be under constant selection in their natural habitat, due to the changing weather between and during each growing season (i.e. varying precipitation and temperature). This fast growing trait (relative to other genotypes), seen to be independent of the photoperiod, temperature and irradiance, could be an adaptive strategy to increase colonization advantage. By being less sensitive towards a particular seasonal cue, the individual would have an increase of tolerance to variation in this cue, and hence be more flexible to grow in a wider range of conditions.

If faster growing plants are fitter in general, it would make sense for a genotype to grow faster in all conditions; all local genotypes would therefore be expected to behave like 5A3 and 10B1 and exhibit fast growth universally. As this does not hold, there must be a reason explaining the variation in growth rate seen among local genotypes. One possibility is that there may be conditions in which 5A3 and 10B1 are at a disadvantage, which were not identified and tested in these experiments. Tradeoffs in performance – negative relationships between traits – are often observed in plant growth, defense and fitness (Kaplan *et al*. 2009; Fine *et al*. 2006; Tian *et al*. 2003). It may be that, in these families, poor growth in *such* conditions is a tradeoff for faster growth in all others. If these conditions occur quite rarely in the natural environment (such as seasonal drought in the Edinburgh area), then it would be harder to identify and assess them when experimenting in the natural environment. Additionally, if such conditions are indeed rare, then poor growth in 5A3 and 10B1 would be of relatively little penalty when factored against superior growth in more common conditions which occur nearly all year round.

Alternatively, there may be disadvantages in growing fast; such as risking nutrient depletion (i.e. some fast growing genotypes produced a low seed yield, or died without reaching their reproductive phase when under controlled conditions), or increased susceptibility to drought (i.e. 10A1 and 10B1 die before reproduction in the field during late spring/ early summer; Chapter 5). This raises the question of whether plants regulate their growth in response to environmental signals or whether they have been selected to maximize growth as far as the conditions will allow. The former view would be more consistent with the idea that there is a cost for fast growth, such that plants restrict growth below the potential maximum rate in order to mitigate these costs. The results in this chapter and the growth outcome of the '10s' family in the field (discussed previously in Chapter 5) suggest that universally fast growth may incur a cost towards reproductive success, as seen in spring 2009 for the fast growing 5A3 and 10B1 individuals.

Proposal of growth strategies in some local genotypes from different locations

Based on growth data from seasonal experiments (and supported by results from controlled experiments), local genotypes from certain location can be said to adopt one of three types of growth strategy; winter annual, summer annual or generalist. Winter and summer annuals grow (relatively) faster in autumn and spring respectively. Generalists show similar growth performance in all seasons, relative to other individuals' growth rates. Observation of relative growth rate across the experimental populations was used to estimate the growth strategy being employed by families, based upon their performance relative to other families in each season.

The nine populations sampled from around the King's Building area contained individuals following each type of growth strategy (i.e. plants from "5" can be classed as generalist, whereas "2" was observed to be predominantly summer annual). Genotypes from location 10 (Loanhead) indicated a preference for generalist behaviour. Locations 11 (Lothianburn) and 13 (Straiton) were observed to be associated with more winter annual behaviour. Only two individuals from location 12 (Straiton) were used in the experiments; these appeared to be summer annuals, although the lack of data makes a conclusion difficult (please refer to maps in Fig. 2-1-1A and 2-1-1B for all locations).

The fluctuation of relative growth rate (RGR)

The RGR of local *A. thaliana* fluctuated with the periodic seasonal temperature changes (Fig. 7-5A). In particular, RGR observed in seasonal field conditions showed the most dramatic fluctuations compared to semi natural (GH) and controlled conditions (Fig 7.5B; 7-6). Photoperiod and irradiance are the least likely factors influencing seasonal RGR due to their consistent annual patterns (i.e. day length gets progressively shorter towards winter and longer towards summer).

The most obvious explanation is that differences in temperature are largely responsible for RGR fluctuations. The changes in RGR mirror those of average daily temperature in field experiments (Fig 7-7). RGR was seen to tail off during the experiments. This was either because of environmental restriction on growth (freezing winter temperatures) or flowering.

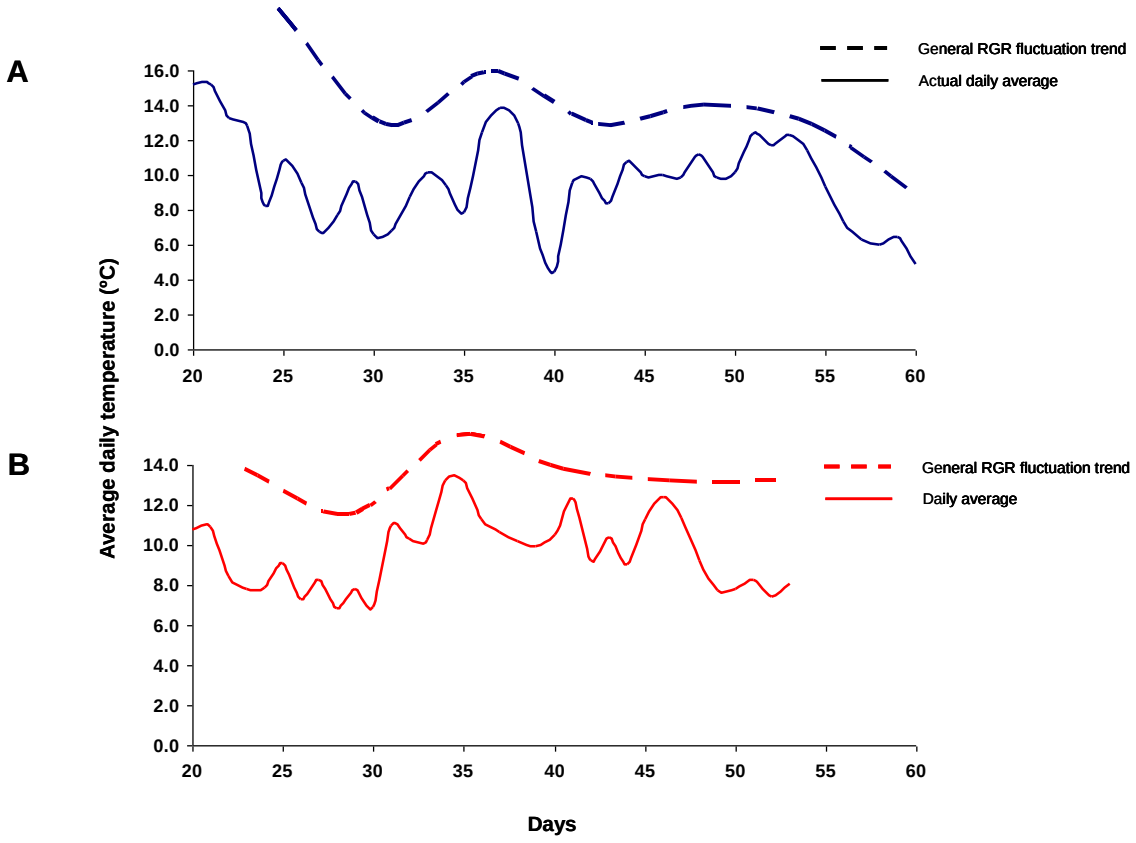

Figure 7-7 **The relationship between RGR and temperature**. Fluctuations in RGR (not drawn to scale) are compared to daily average temperatures for autumn (**A**) and spring (**B**) in the field.

The fluctuation of heritability (H^2)

The heritability of RGR varied over time. It is not unexpected that H^2 decreases with age, as non-genetic factors are likely to have an increasing influence upon phenotype as time progresses. Similar fluctuation of H^2 was reported by Wu (1972) who measured fresh weight of *A. thaliana* F1 hybrids under different controlled environments and found high H^2 in early development.

The unexpected increases of H^2 values in later vegetative growth could be attributable to the transition to reproduction. Growth in rosette area stops as plants flower. Since flowering time is highly heritable, genetic differences between families in flowering time genes could contribute to differences in the estimates of rosette area growth.

In summary, these controlled experiments have provided information on the similarities and differences of the effects of photoperiod and temperatures upon growth: providing a context to those recorded in natural seasonal experiments (Fig. 5-1-4, Chapter 5). The consistent fast or slow growth rankings of the same genotypes in both controlled and natural experiments support the suggestion that different environmental factors have a combinatorial effect upon phenotypes, as observed in the field. Conversely, a few minor differences between controlled and natural outcomes suggest that other factors, such as water availability (i.e. drought tolerance), might have an effect in the natural environment.

Chapter 8 Seasonal morphologies in local *A. thaliana*

Introduction

Differences in plant morphology can be genetically determined, influenced by exterior factors (plasticity) or a combination of both. Where they have a genetic component, the differences can be adaptive. For example, in bambuseae and cactaceae families different morphologies are assumed to have evolved as adaptations to their different native habitats. Bamboo (a grass species), probably evolved to become a more successful competitor in tropical rainforests through developing woody tissue and growing long stalk stems to adopt the form of trees (Clayton 1981). In contrast, cacti species have adapted to dry desert environments; growing fat waxy stems designed to retain water, and spikes (instead of leaves) to reduce water loss by transpiration and aid protection.

Plants display phenotypic differences in response to developmental signals and environmental cues, such as change of organ colour, shape and architecture. This plasticity might be under genetic control. For example, Pigliucci *et al*. (1995) investigated the reaction norms (the phenotypic expression pattern of a genotype across different environments) of four *Arabidopsis thaliana* populations in response to three different environmental gradients (light, nutrients and water). They measured nine phenotypic traits (i.e. number of leaves, flowering time, inflorescence height, number of branches etc.) and detected a substantial amount of genetics variation for plasticity in response to different light and nutrient treatments.

A. thaliana, which occurs across a wide range of habitats, exhibits a variety of morphological forms which may be a result of adaptations or plastic responses to local conditions. These morphological characters may influence the overall success of a plant within its local habitat. Consequently, the genetically determined variation of morphological traits has been studied in *A. thaliana* and other plant species, including crops (reviewed by Alonso-Blanco *et al*. 2009). Several studies on domesticated traits

(i.e. the shape and size of fruits and cereal grains) have identified numerous quantitative trait loci, QTL, underlying these traits and several QTL genes have been isolated (reviewed by Alonso-Blanco *et al*. 2009).

Leaf morphology and rosette architecture

Leaves are vital organs due to their role in both photosynthesis and transpiration, and optimal leaf morphology and architecture for local conditions might provide a fitness advantage. Leaves of annuals such as *Arabidopsis thaliana* are determinate organs, in that they only grow during a limited time period over the life of the plant. Consequently, it is probably important for *A. thaliana* leaves to be highly plastic during growing season, so as to allow growth in a wide range of local conditions.

Studies have shown that differences in leaf morphology can be genetically controlled but influenced by local environmental factors such as water availability, light levels and temperature fluctuation (reviewed by Tsukaya 2006). Cookson and Granier (2006) studied the effects of light intensity at cellular, leaf and whole plant levels. They discovered that plants which experienced shading had both reduced leaf initiation rates and leaf expansion rates. However, the duration of expansion for each leaf increased, potentially allowing larger final leaf sizes than under optimal light condition. Epidermal cell division rates were decreased, but cell size increased in shaded plants; this can explain differences in final leaf size under shading.

Other leaf morphological changes associated with photosensitivity include hypocotyl elongation, petiole elongation and leaf inclination; the latter two of which can lead to a change of rosette architecture. Plants respond to light signals using photoreceptors in leaves, including red and far red light absorbing phytochrome, blue light cryptochrome and phototropins. Research has indicated that morphological changes in response to light may convey a fitness advantage in *A. thaliana*, and that variation in the responses might be genetically determined and involved in local adaptation. Maloof *et al*. (2001) studied

hypocotyl elongation and found that different accessions responded differently to all light and hormonal conditions tested. They found a modest inverse correlation between hypocotyl length in white light and the latitude of origin of the accessions, consistent with adaptation. They speculated that seedlings, which are on average taller at lower latitude (closer to the equator), are adapted to high light intensity through reduced light sensitivity. They also suggested that variation in hypocotyl length may results from "hard-wired" genetic differences in developmental programs, rather than from changes in light-response.

Anthocyanin

Foliar anthocyanin productions have been documented in a wide variety of plants species in various environments. It is generally viewed as a response to environmental stresses which may confer an advantage in certain conditions (reviewed by Chalker-Scott 1999). Maple, dogwood and oak trees, for example, produce anthocyanins in leaves prior to autumn leaf shed. It has been hypothesized that metabolic changes during leaf senescence increase susceptibility to light-induced oxidative leaf damage, and that anthocyanins are produced as protective agents. Feild, Lee and Holbrook (2001) speculated that anthocyanin production in Red-Osier Dogwood (*Cornus sericea*) may also act as cost-effective protection against oxidative damage by both reducing the light absorbed by free chlorophyll and absorbing oxygenated radicals produced by after light absorption by free chlorophyll.

Hoch *et al*. (2003) tested this hypothesis by comparing wild-type and anthocyanin deficient mutants of the woody species, *C. sericea*, *Vaccinium elliottii* (Elliott's Blueberry) and *Viburnum sargentii* (Tianmu Viburnum). They found wild-type plants had a higher photosynthetic efficiency than their anthocyanin deficient mutant counterparts in natural outdoor autumn conditions or high stress growth chamber conditions (10 hour day light, high light: 900 µmol m^{-2} s^{-2}, low temperature: 3ºC day to 2ºC night). Under low stress chamber conditions (10 hour day light, high light: 380 µmol

m^{-2} s^{-2}, low temperature: 20ºC day to 4ºC night), wild type and mutant plants had similar efficiencies. Wild-type plants were also able to recover after transfer from high to low stress conditions and to complete leaf senescence, whereas mutant plants shed their leaves while still green. As a consequence, wild type plants that were able to produce anthocyanin had higher foliar nitrogen resorption during leaf senescence.

Unlike the woody species used by Hoch *et al*. (2003), *A. thaliana* does not shed its leaves and growing close to the ground means a greater chance of growth in shaded conditions in its natural habitat. However, a chamber study by Noodén *et al*. (1996) showed that *A. thaliana* (*Columbia-0*) exhibited increased anthocyanin production in a lower light intensity than studied by Hoch *et al*. (2003). They found a significant loss of chlorophyll and increase in anthocyanin concentration when plants grown under long day full light conditions (300 µmol m^{-2} s^{-2}) compared to those grown under long day reduced light conditions (180 µmol m^{-2} s^{-2}). More recently, Diaz *et al*. (2006) hypothesized that leaf yellowing and an increase in anthocyanin in *A. thaliana* are genetically independent responses to nutrient deficiency. They propose that yellowing is a strategy to concentrate available resources in younger leaves, by promoting senescence and chlorophyll breakdown in older leaves. Anthocyanin accumulation could then be a protection against light sensitivity during senescence.

Fasciation

Fasciation is a spontaneous abnormal organ enlargement in plants during growth development. It was initially studied as a morphological change, before attention turned towards a genetic basis around the end of the 19th century (Hus 1906; Mertens and Burdick 1954). Fasciation can occur in the stems, fruits and flowers of plants grown in the wild, in crops such as sweet peas and tomatoes. Earlier records have reported fasciation being observed in over a hundred plant species (White 1948).

The cause of fasciation in wild or domesticated plants is not immediately obvious, due to the variation in the conditions under which it has been observed. However, fasciation has been seen to be induced by applications of plant hormones (auxin, cytokinin), wounding, or changes in seasonal weather (i.e. transition from winter to spring). Fasciation can also be caused by infection; Thimann and Sachs (1966) reported a bacterial (*Corynebacterium fascians*) infection induced fasciation in garden pea, possibly through cytokinin produced by the infectious organisms.

Advances in molecular genetics in the last few decades have revealed how fasciation might result from mis-regulation in apical meristems (Karakaya 2002). To balance a pool of undifferentiated meristem cells and their differentiated daughter cells involves a complex regulation and signaling mechanism: an error in the process may result in an increase in the size of the meristem and fasciation.

For example, through molecular characterization of *A. thaliana clv* mutants, Clarke *et al*. identified a signaling pathway in the shoot apical meristem that is dependent on *CLAVATA* (CLV) genes (reviewed by Clarke 2001). A pool of stem cells at the centre of the shoot apical mersitem expresses the *CLV3* signaling peptide, which is perceived by a receptor kinase complex containing *CLV1* and *CLV2* in more peripheral cells. As a result of this signaling, expression of the stem-cell promoting *WUSCHEL* (*WUS*) transcription factor gene is reduced. Loss of any of the CLV gene activities therefore leads to an increase in the stem cell population and an increase in the size of the shoot apical meristem, which can lead to fasciation. Another study by Clarke *et al*. (1996) reported 'cross talk' between the *CLV* signaling pathway and the *SHOOT MERISTEMLESS* (*STM*) gene, which is required for embryonic shoot meristem formation. They found that *stm* mutations enhanced the fasciated postembryonic shoot and floral meristem effects of *clv* mutations and proposed that a balanced quantitative relationship between *CLV* and *STM* is essential to maintain proper shoot and floral meristem development.

Fembrini *et al*. (2006) found that a recessive mutation in the *STEM FASCIATED* (*STF*) gene caused multiple fasciation features in *Helianthus annuus* (sunflower); i.e. can affect

SAM shape, stem diameter and inflorescence size. They speculated that *STF* may play multiple roles in plant development. They also found that *stf* mutants produce higher level endogenous auxin that could have potential roles in altering morphology.

In a study of the functions of the *AtBRCA2a* and *AtBRCA2b* genes in *A. thaliana* somatic cells, Abe *et al*. (2009) found that 5 to 40% of double *atbrca2* mutants displayed stem fasciation and/ or abnormal phyllotaxy. *AtBRCA2* genes are related to *BRCA2* (Breast Cancer Susceptibility gene 2) from mammals, belonging to a tumour suppressor gene family. In mammals, BRCA2 ensures efficient double-strand DNA break repair via homologous recombination and a mutation in *BRCA2* can result in uncontrolled cell growth. Abe *et al*. reported that cell cycle progression in the double *atbrca* mutant was altered and that the number of *atbrca2* (single or double) mutant plants exhibiting fasciation increases after γ-irradiation treatment, which breaks DNA, suggesting that fasciation might result from unrepaired DNA breaks.

This chapter presents the morphological observations made during previous seasonal growth studies. Growth in the natural environment, with combination and variation of environmental factors, means that morphologies may occur which are not necessarily seen or reproducible within a controlled growth environment. Consequently, changes in morphology of plants grown in the field (LE and HE) and in colouration were noted whilst growth rate and flowering time were being measured. It was anticipated that morphological changes might suggest when non-climate factors were affecting growth performance – for example if disease was stunting growth, or if unseen predators or parasites were damaging the plant.

A number of morphological characteristics of GH, LE and HE were recorded. Within these characteristics, leaf colouration, rosette architecture and inflorescence morphology were found to be associated specifically with climate patterns in autumn and spring. These changes have not been previously recorded in studies of wild *A. thaliana* under natural conditions.

These local *A. thaliana* have shown consistent growth performance that suggested adaptation to seasonal environmental conditions. Morphology is known to play a role in plant adaptation: it is a logical next step to consider how morphological differences might affect a plant's performance in a natural environment. It is possible that different adaptations (adaptation to different environments or seasonal conditions) might be indicated by different morphological responses to the environment.

Results

The morphological changes in rosette architecture and leaf colouration in field plants were noted primarily for the purpose of plant health documentation, with an additional view towards identify possible adaptive traits. Any change of rosette shapes from a typical flat arrangement of fan like leaf blades and petioles was noted. Examples of typical *A. thaliana* rosette morphology are shown in Figure 8-1A & B. Weekly photographic images were compared within and between sites and seasons. Shapes of inflorescences stems were also noted in the first spring, when fasciation was observed.

Seasonal rosette architecture transformation

A distinctly different rosette morphology was observed in autumn and spring plants grown in the field (LE and HE), compared to GH (Fig. 8-1; 8-2; 8-3). There were also differences observed between autumn and spring groups at each site. All young seedlings at different sites started off with similar rosette morphology, through the autumn season (September to November): round leaf blades with short but visible petioles (Fig. 8-1A, C, F & I; 8-2A & D).

The rosette morphologies of field and GH plants were observed to progressively change during winter (from the beginning of December) into notably different shapes. Leaves of GH plants rapidly expanded in a relative proportionate manner, where leaves and petiole

both grew simultaneously: though rosette areas of adult plants increased the architecture of the rosette remained relatively flat (Fig. 8-1B). In the field, adult plants from autumn LE and HE in 2007 and 2008 grew into a 'mushroom cap' shape, as petioles of newly formed leaves hardly developed and those of the older leaves did not elongate as much as plants grown in GH (Fig. 8-1D, E, G & H; 8-2 B & C). As a result, the newly formed leaves of field plants appeared to stack on top of the older leaves. This created a rosette structure with depth, resembling the shape of a mushroom cap (Fig. 8-1D; 8-2B).

However, this mushroom cap rosette architecture was observed neither in LE nor HE plants grown in autumn 2009 (Fig. 8-1J, K; 8-2E, F). The general rosette shape of most field plants in autumn 2009 (the warmest autumn of three experimental years) was observed to be very similar to those grown at GH in the past two autumns – flat rosette with typical fan like leaf blade and petioles. Rosettes of late flowering plants in this autumn were more densely packed than in the earlier flowering ones: the leaf blades of leaves formed over winter period appeared to become more elongated compared to the plants grown in the previous two autumns. This suggests the transformation of rosette architecture in the field is highly likely to be linked to seasonal weather, such as temperature.

In addition, the mushroom cap rosette architecture of autumn plants in 2007 and 2008 was relatively well preserved until spring (with little leaf expansion) except in late flowering families (i.e. 5A3, 10A1 and 10B1). Early flowering families such as 9A2 and 6A3 were observed to retain the mushroom cap architecture until flowering and senescence. In contrast, families that flowered later tended to show more leaf blade elongation: changing the rosette into a more chrysanthemum flower like morphology (picture images not shown). This suggests that late flowering may have provided an opportunity to resume leaf growth.

In contrast to autumn, field plants in spring were observed to have little transformation in rosette architecture (Fig. 8-3). Although the average rosette size was observed to be smaller than GH cohorts, the general shapes of leaves and rosette architecture of plants grown at LE and HE were similar to those grown at GH.

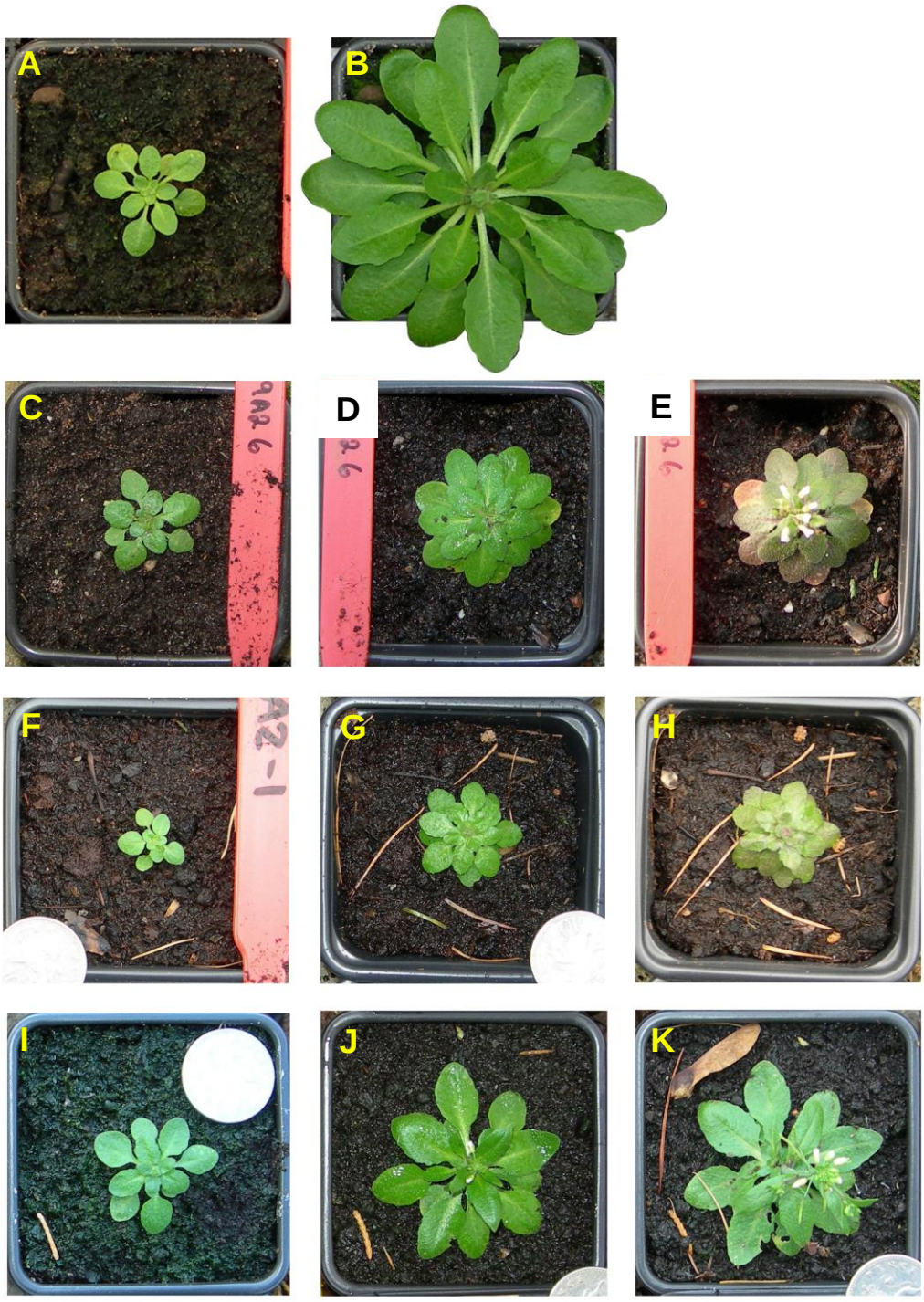

Figure 8-1 **Rosette shapes and architectures of autumn plants grown at GH and LE.** Illustration of family 9A2 rosette shapes at GH (**A** & **B**) compared to LE (**C** – **K**) from three autumns: autumn 2007 (**C** – **E**), autumn 2008 (**F** – **H**) and autumn 2009 (**I** – **K**). 'Mushroom cap' rosette architecture was observed during winter months

(January to February) in autumn 2007 and 2008 sown plants. Mushroom cap rosette was not observed in autumn 2009, although the younger plant (**I**) grew to a similar shape as its autumn 2007 equivalent (**C**).

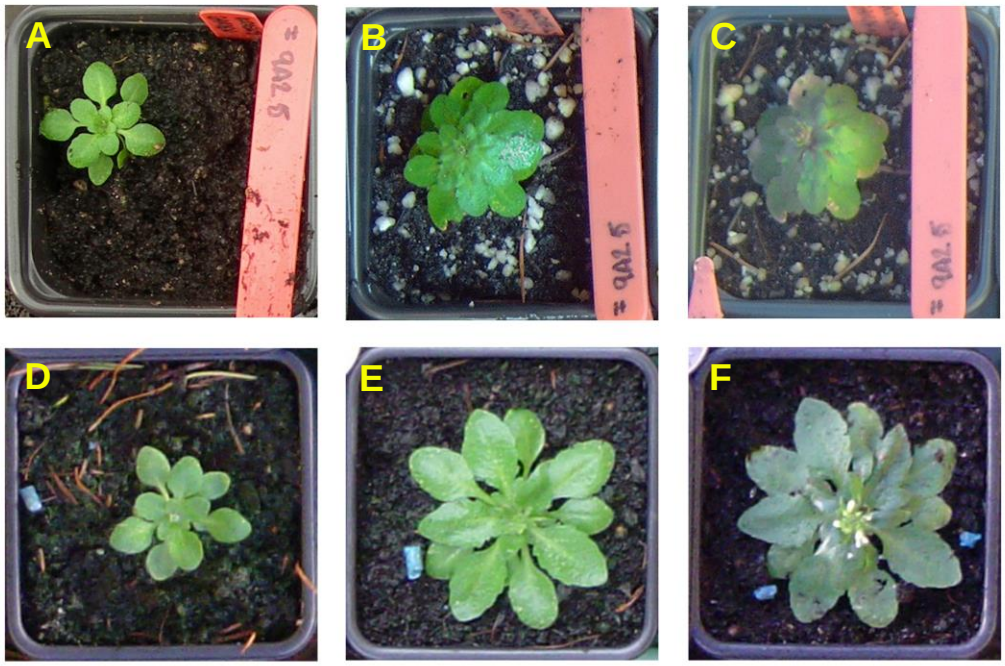

Figure 8-2 **Rosette shapes and architectures of autumn plants grown at HE in autumn 2007 (A – C) and autumn 2009 (D – F)**. Autumn HE plants grew similar shapes and architectures as LE cohorts (Fig 8-1**C – E**, **I – K**).

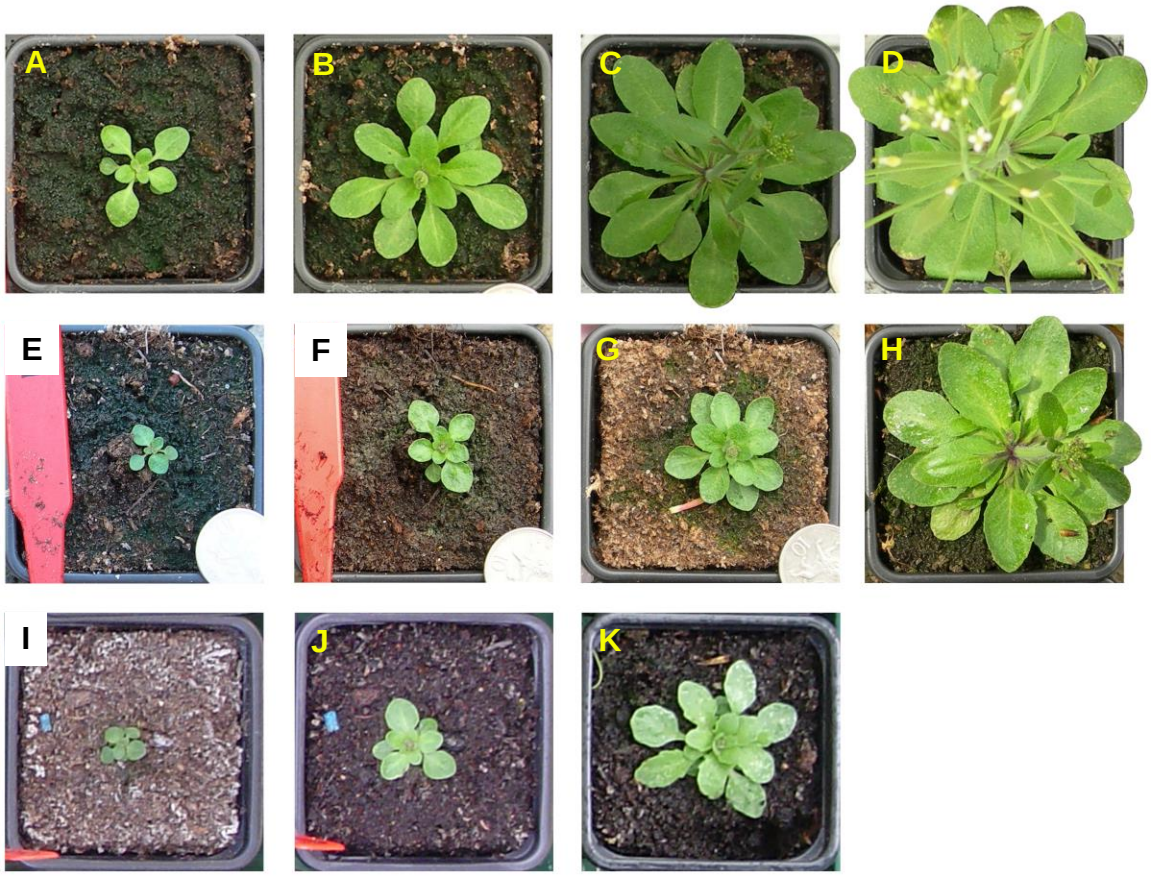

Figure 8-3 **Rosette shapes of spring plants grown at GH (A – D), LE (E – H) and HE (I – K)**. Mushroom cap rosette was not observed in spring LE and HE plants. Spring LE, HE plants grew similar rosette shapes to GH plants in previous years. Plants in the above illustration are family 9A2.

Seasonal anthocyanin accumulations

Various levels of anthocyanin accumulation were observed across all plants grown in the field (LE and HE) in autumn and spring. Plants grown at GH in autumn and spring were observed to remain lush green throughout their vegetative phase until entering the reproductive stage (photo images not shown).

Autumn plants exhibited very pronounced anthocyanin accumulation. Both LE and HE plants produced increasingly higher levels anthocyanin through the winter months, correlating with decreasing temperature. This gradually strengthened to a reddish-purple

colouration across the entire leaf area, becoming prominent at the end of winter (February) (Fig. 8-4A – E). Most plants were observed to gradually lose this strong reddish purple hue in early spring prior to bolting (the beginning of inflorescence growth), as temperature rose and day length increased.

In addition to losing anthocyanin, families from locations 5, 10, 11 and 12, were observed to have recovered green pigmentation in their leaves in early spring (mid March) (Fig. 8-5G – I). This recovery, however, only lasted approximately one week, after which anthocyanin accumulation began once more prior to flowering (Fig. 8-5J). It is possible that the second accumulation of anthocyanin occurred at leaf senescence associated with flowering.

In contrast, spring LE and HE plants showed less dramatic anthocyanin colouration. Plants grown at LE and HE in spring were also observed to have a slightly different anthocyanin accumulation patterns. Instead of a drastic colour change towards the end of their life cycles, anthocyanin accumulation occurred at two different stages of vegetative growth. Firstly, it was observed at the shoot apex in newly formed leaves during early juvenile development, beginning after approximately three to four weeks growth in the field. It was interesting to note that some families (1, 6, 8 and 13) had slightly more intense colouration on their new leaves than the remaining individuals from the populations, suggesting that differences in the response are genetically determined.

This colouration gradually decreased over time during the rapid vegetative growth phase. Examples of the transient changes of anthocyanin accumulation (1A5 has been used in the illustration) in spring are shown on Figure 8-4 F to J: the level of anthocyanin gradually decreased during the rapid vegetative growth phase. Spring plants did eventually show an increase in anthocyanin accumulation just prior to flowering, starting from the tips of the older leaves and the petiole ends of the younger one. These later colour changes were more pronounced in certain families, including individuals from 1A5, 1B2, and 1E1. Families 9A2 and 9A3 were observed to have little anthocyanin

accumulation at flowering compared to other families. Most other plant families were observed to have visible anthocyanin colouration around the start of senescence except a few families originated from locality 1 (1A5, 1B2, and 1E1), which started anthocyanin accumulation long before senescence.

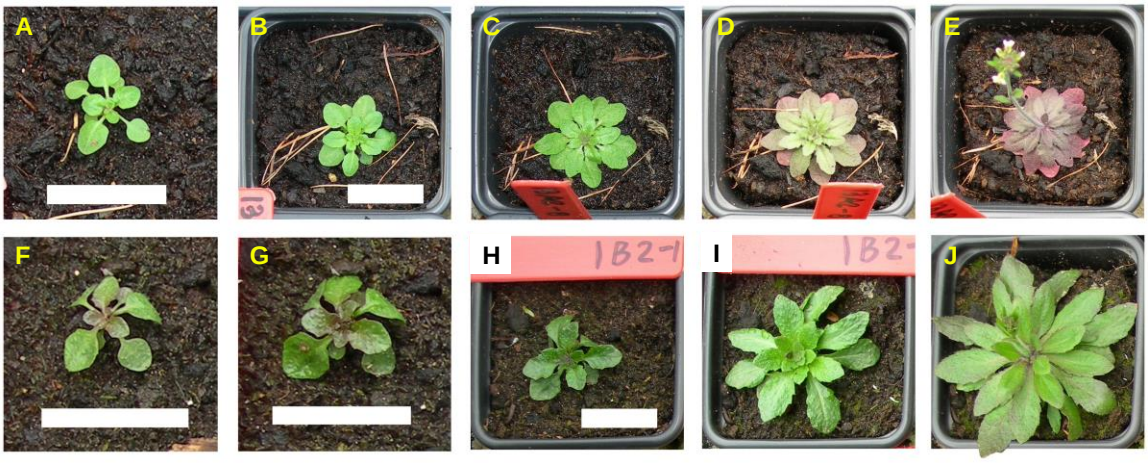

Figure 8-4 **Anthocyanin accumulations in autumn** (**A** – **E**) **and spring** (**F** – **J**) **2008**. All autumn LE (and HE) plants developed drastic color change between February and March, especially at fruiting (**D** & **E)**. All spring (LE and HE) plants displayed various environmentally transient anthocyanin accumulations (**F** – **J**). Anthocyanin accumulated at the shoot meristem (centre) of juvenile plants (**F** & **G)**, gradually disappeared (**H** & **I**), and returned at flowering (**J**). White bars represent a length of 24.5 mm, derived from a fixed size reference. Pictures of developing autumn plants were selected at one month intervals, beginning with November, and at one week intervals for spring plants from mid April to May.

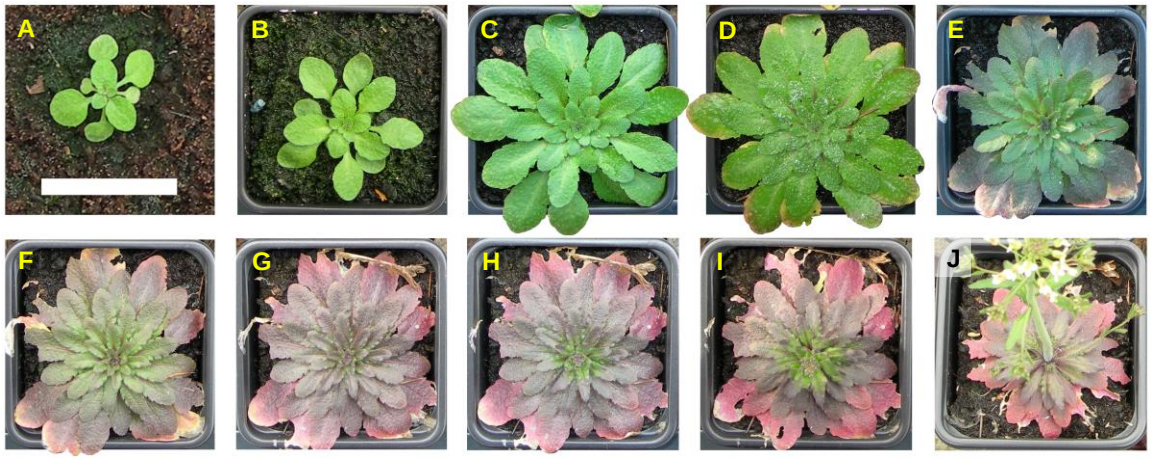

Figure 8-5 **Transient anthocyanin accumulations in autumn adult plant at LE (A – J)**. No noticeable anthocyanin accumulation was observed at the shoot apex of juvenile plants (**A**). All autumn (LE and HE) plants developed strong colouration, where anthocyanin accumulation began to increase gradually over three weeks (**E** – **G**). The plant(s) have partially recovered green colour at the shoot meristem briefly in early spring (**H** – **I**) before returning to purple at flowering. White bars represent a length of 24.5 mm, derived from a fixed size reference. Pictures of developing autumn plants (family 5A3, example of illustration) from **A** to **E** were selected at one month intervals, beginning with September and ending with January. Pictures **F** to **J** presented above were taken on 12th February, 17th February, 12th March, 17th March and 23rd March respectively in 2009, to give the best representative illustration.

Anthocyanin accumulation in growth chamber

Similar to the seasonally grown plants, significant anthocyanin accumulations were observed in one of the growth chamber experiments set up to investigate the effect of abiotic variables on growth rate. In the 5ºC LD growth chamber, most but not all lush green plants were observed to change colour, between approximately 20 to 25 weeks of age. Anthocyanin production began either in cauline leaves or the edges of the rosette leaf blades. These colour changes were permanent; the plants did not return to a green colour at any later developmental stages but continued to accumulate anthocyanin until senescence.

Depending on genotypes, plants exhibited one of three responses; 1) accumulating a high level of anthocyanin (intense purple) prior to bolting, 2) accumulating anthocyanin soon after senescence begins (yellowing at the tip of leaf blades) at flowering or 3) having no visible accumulation of anthocyanin at flowering nor when senescing began. These examples are shown on Figure 8-6. Family 5A3 was one of the few families that accumulated high level of anthocyanin. Its rosette leaves were intensely purple at flowering and the onset leaf senescence was observed near the end of fruiting. In contrast, rosette leaves of families 9A2 and 11s (11A2, 11A5 and 11B2) remained lush green until flowering. Anthocyanin accumulation occurred when older leaves in families 11s were observed to enter senescence, soon after flowering, whereas no anthocyanin was seen on rosette leaves of 9A2, even when senescence started in the middle of fruiting stage.

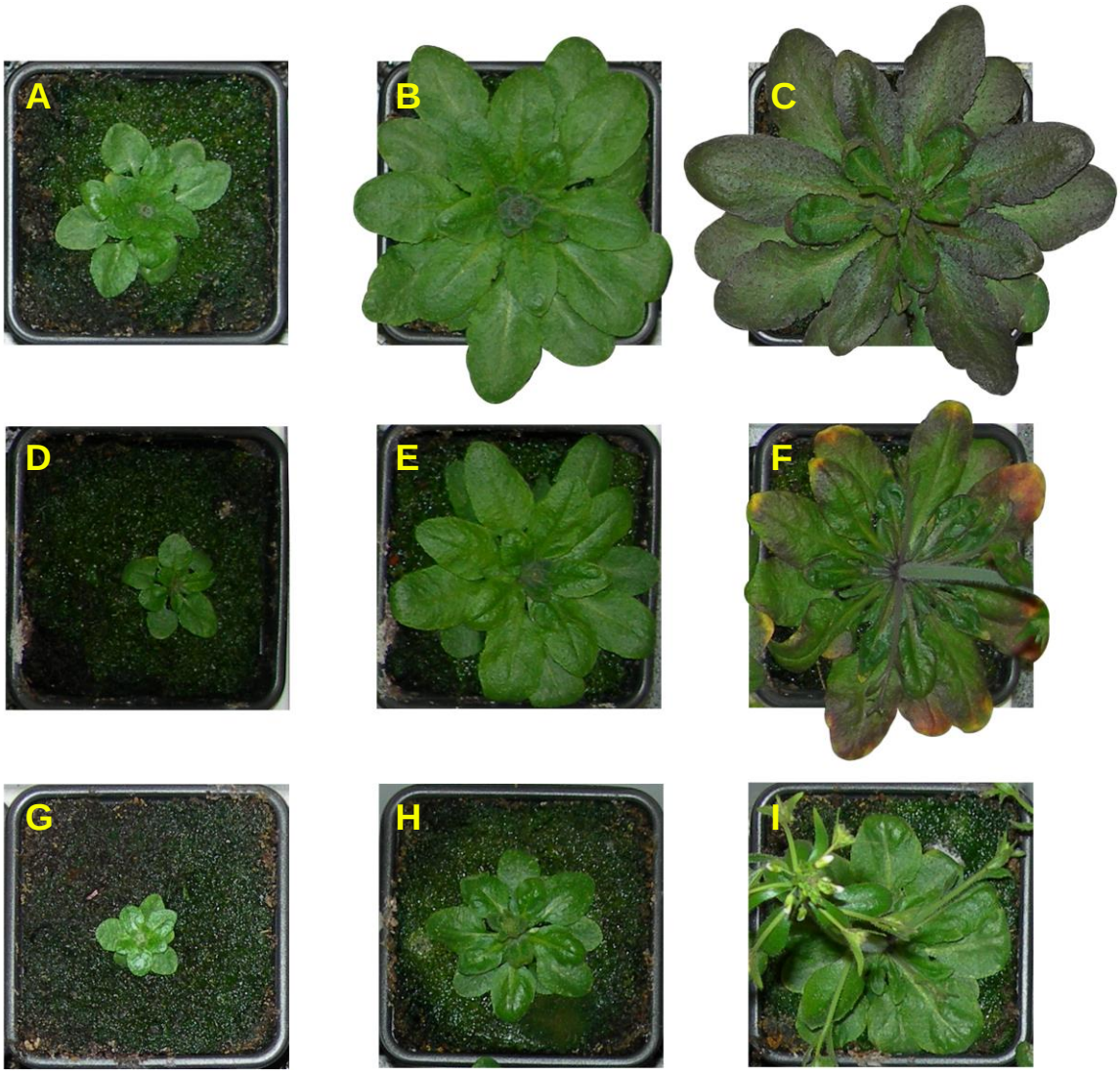

Figure 8-6 **Anthocyanin accumulation in a 5ºC LD chamber experiment**. Different genotypes: 5A3 (**A** – **C**), 11A5 (**D** – **F**) and 9A2 (**G** – **I**) show different levels of anthocyanin accumulation. Adult 5A3 has accumulated noticeably high level of anthocyanin at bolting (**C**). Compared to 5A3, 11A5 accumulates intermediate level of anthocyanin with onset of senescence. Neither anthocyanin accumulation, nor senescence was noted on 9A2 at flowering. Pictures of developing plants were selected at one month intervals.

Inflorescence fasciation

Different width and rigidity of inflorescence stems was observed in plants grown in seasonal experiments and under controlled conditions: local *A. thaliana* grew woodier

inflorescence stems compared to laboratory line, *Col-0*, when grown in the field or controlled growth room (i.e. 22ºC LD or SD) conditions (Fig. 8-7). In addition, the thickness and rigidity of the inflorescence stems varied across different sites and seasons.

The inflorescence produced by GH plants grown in autumn (around 10ºC average daily temperature), were noticeably thicker and taller than those grown in the field. The main inflorescences stems at GH in autumn were measured 2.89 ± 0.022 mm (mean ± SE, n = 111) in diameter (SE) and over 30 cm height (at harvest in mid April). At LE and HE, the main inflorescence stems were rigid and measured approximately between 0.8 – 1.5 mm in diameter (data not shown).

In spring, most plants at LE and HE were observed to grow slightly thicker (by approximately 0.3 mm) main inflorescence stems than the autumn cohorts. Spring GH plants develop a similar thickness of main inflorescence stems as the GH autumn cohorts. Half way through flowering, some spring GH plants (early or late flowering) were observed to grow fasciated auxiliary inflorescences in early summer that resembled tagliatelle pasta with flower heads similar in appearance to broccoli crowns (Fig. 8-7E). Fasciation of axillary inflorescences was limited almost exclusively to certain families. Of the 23 families grown in GH spring 2008, seven contained plants that developed fasciated auxiliary stems. This corresponds to 13% of individuals in the GH population (n = 69). In spring 2009, 3 out of 19 families exhibited the phenotype. Although only 4% of the population (n = 95) exhibited this phenotype, these were the same sub group of families - 5A3, 11A5 and 12A1 - that have previously displayed fasciation in spring 2008 (Table 8-1). Each of these plants grew at least one fasciated auxiliary stem and some individuals from 5A3 and 12A1 grew up to three fasciated auxiliary stems. This suggests that the susceptibility to fasciation is genetically determined.

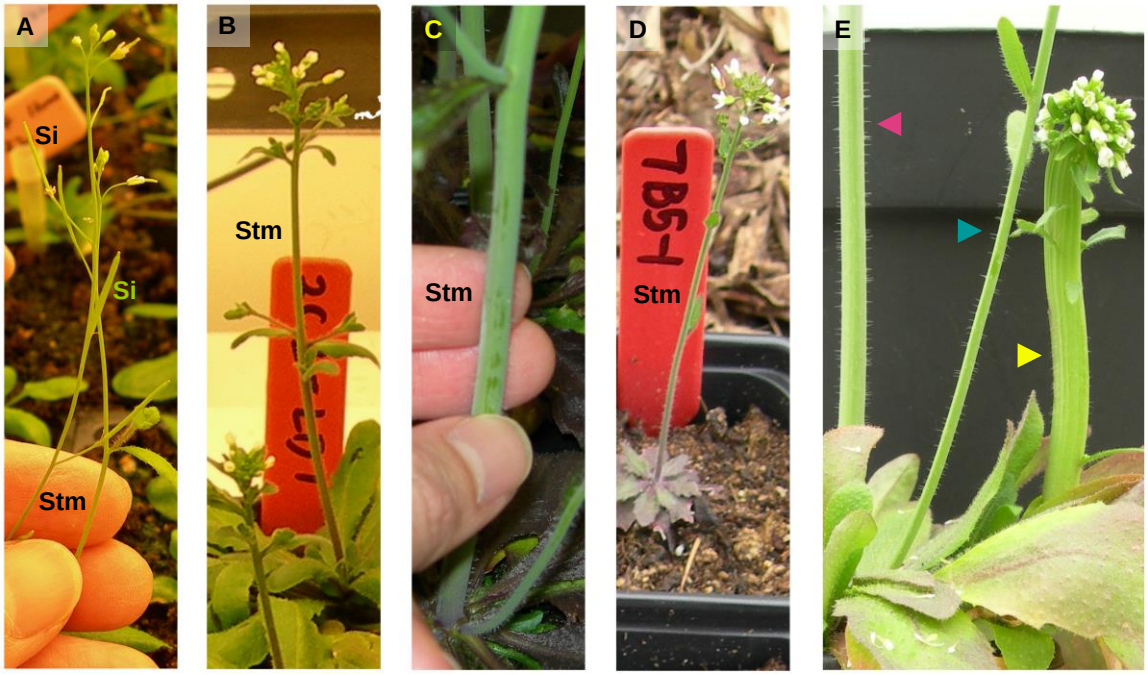

Figure 8-7 **Inflorescence stems and fasciation in seasonal plants**. Inflorescence stems (**Stm**) of *Col-0* (**A**) and wild *A. thaliana* (**B**, 2C2) in a 20ºC LD growth room, 5ºC LD growth cabinet (**C**, 1B5), at LE (**D**, 7B5) and GH (**E**, 5A3). *Col-0* is used for a visual comparison of stem thickness; the stems are nearly as narrow as the siliques (**Si**). Wild *A. thaliana* (**B** – **E**) grew thicker main inflorescence stems. An example of fasciation observed in GH (**E**); the thick woody and rigid main inflorescence stem is shown with a red arrow, a normal auxiliary stem with a green arrow and a fasciated axillary stem with a yellow arrow.

	Spring 2008	Spring 2009
1D1	1	0
2C2	1	0
2E1	1	0
5A3	2	1
6A2	1	N/A
11A5	1	1
12A1	2	2
Total fam	*23*	*19*
Replicates	*3*	*5*

Table 8-1 **Summary of the number of spring plants in GH that produced fasciated auxiliary inflorescence**. The total number of families (***Total fam***) grown in GH 2008 and 2009 are 23 and 19 respectively, with 3 and 5 replicates of individuals per family. 6A2 was not grown in spring 2009.

Discussion

The observations presented in this chapter illustrate a visual plasticity response displayed by local genotypes in the field during different seasons and conditions. Amongst all the recorded field environment variables, a relatively stable annual humidity pattern (Chapter 4) indicates that it was least likely to have a direct effect on growth or morphology of wild *A. thaliana* in the natural environment. In addition to external stress signals, genotypes could differ in their environmental responses, either through organ shapes or colour, indicating that genetics may have an influence on the exhibition of these morphologies.

Phenotypic plasticity in rosette shape

The changing environmental conditions had different influences on rosette architecture displayed by local genotypes. The transformation from very similar looking rosette shapes at all three sites and for all genotypes initially, to a strikingly different shape for plants grown at LE and HE, strongly suggests that environment is responsible for this variation. Because humidity varies little between experiments and day length and light intensity are approximately equal at LE and GH within the same experiment and GH plants do not show variation in rosette architecture, it seems likely that architecture changes in response to temperature.

The mushroom cap rosette observed over winter (December 2007 to February 2008, and December 2008 to February 2009) might be an adaptive response to cold. Perhaps changing the relatively flat architecture to a structure with more depth could allow young rosettes to preserve core thermal stability through periods of low temperature, so growth can resume when better conditions arise. The resumption of leaf growth (elongation and expansion) in most field plants, particularly late-flowering families, in spring led to yet another transformation of rosette shape. This further highlights the possibility that these

plants might have adopted architectural transformation strategies to survive temporary inclement conditions until spring. It is also possible this final transformation maximizes surface area, to take advantage of the spring increase in light resources.

A second possibility is that the mushroom cap shape was simply the result of reduced growth in the winter environment. Day length and temperature gradually decrease in autumn. Growth was observed to be slow but possible in a 5ºC SD controlled chamber, simulating a short cold late autumn/ early winter days. Although this supports the possibility of some limited growth during winter, winter temperature could be lower than this (average min 1ºC to 2ºC, average max 6ºC to 8ºC; Chapter 4), suggesting that low temperature might effective prevent leaf growth for much of the winter. It is not clear whether the small leaves of the mushroom cap architecture were initiated during in the winter, in which case leaf growth might be more sensitive to low temperature than leaf initiation, or whether low temperature inhibits both leaf initiation and leaf growth to a similar extent, in which case the small leaves of mushroom cap plants might have been initiated earlier in the autumn.

Observations taken in the warmer autumn of 2009 further illustrates how environmental conditions can be seen to have induced morphological change. Conditions during this autumn were relatively mild (average min 4ºC to 10ºC, average max 9ºC to 16ºC; Chapter 4); this corresponded with a reduced morphological change over winter. Although winter (December 2009 to February 2010) was the coldest in three experimental years (average min 0ºC to 2ºC, average max 4ºC to 6ºC; Chapter 4), the more hospitable conditions in autumn had allowed for a greater degree of rosette establishment through leaf growth. It is possible that after a certain level of rosette and leaf establishment, that these became less plastic (with regards to morphological changes).

The relatively high and stable level of resource availability and temperature during spring prevents the dramatic transformation in rosette architecture of field plants that was seen in autumn cohorts. If the autumn and winter transformations were survival strategy for

cold weather, it would be expected that this would not be observed (or, indeed, necessary for the plants) in the warmer spring. The variation between autumn and spring grown plants serves as a good demonstration of the plasticity of these local *A. thaliana*, growing at the same locations but under contrasting environmental conditions.

Variation of anthocyanin production

Plants produced anthocyanin at different stages during autumn and spring at both GH and field sites. The timing of accumulation was likely associated with environmental factors, developmental signals, or a combination of both.

Most anthocyanin accumulations from autumn LE and HE plants were partly transient (purple with partially green recovery). The anthocyanin colouration gradually appeared and intensified over the winter months, and then either reduced or persisted in spring. Finally, the reddish purple colour could continue to develop until the beginning of senescence. This supports the suggestion that anthocyanin production in autumn field plants involved both environmental and developmental signals.

Environmental stress is known to induce anthocyanin production in many plant species other than *A. thaliana* (Diaz *et al*. 2006; Feild *et al*. 2001). The first onset of anthocyanin occurrence in autumn grown plants could induced by the various environmental stresses, including prolonged low temperature and lack of water availability during winter months. The freezing temperature and corresponding liquid water scarcity could result in freeze-induced dehydration. Plants may respond to cold-induced leaf tissue damage through production of anthocyanins to protect against transpirational losses. This could entail the use of resources normally either utilized in, or preserved for, growth (reviewed by Chalker-Scott 2002).

Although the intensity of sunlight declines in autumn, plants might likely to experience higher light intensity than in spring because of loss of overhanging deciduous foliage.

Various protective roles of anthocyanins have been suggested and demonstrated (Hatier and Gould 2009). One well documented suggestion of the protective role of anthocyanins is that of photoprotection (Hoch *et al*. 2003; Feild *et al*. 2001). Light-filtering effects of anthocyanins have been shown to expedite photosynthesis recovery (Hatier and Gould 2009).

Most autumn plants were observed to have a short period of recovery in which they produced green leaves. The persistence and further accumulation of anthocyanin during the last stages of autumn plants' lifecycles could be attributed to the onset of flowering and senescence, as seen in most *A. thaliana* grown under controlled conditions. Anthocyanins might function to prevent photo-oxidative damage from free chlorophyll during senescence, as proposed for leaves of deciduous species (Hoch *et al*. 2003).

Spring field plants were observed to exhibit environmentally transient anthocyanin production. Many seedlings and juvenile plants in spring experiments accumulated anthocyanin at two developmental stages: at the shoot meristems in young seedlings and during reproduction. Early developmental anthocyanin accumulation have been seen to occur in many species of plants, notably in tropical plant species when sprouting shoots in spring-like conditions (reviewed by Chalker-Scott 2002).

Hughes *et al*. (2007) studied anthocyanin production and degradation in three tree species, *Liquidambar styraciflua* (sweetgum), *Acer rubrum* (red maple) and *Cercis canadensis* (Eastern redbud). Their findings suggest that anthocyanin production during early stage leaf growth was beneficial. They speculate that anthocyanins serve as protection for young developing tissues before other photoprotective mechanisms are established. Other reports present similar indications that anthocyanin produced at early developmental stage is likely to be linked to photo-induced stress: the pigments would act as a photo-protective shield for young developing organs against damage by UV light (reviewed by Chalker-Scott 2002).

This is a potential explanation for the colouration of young plants' apices in the spring experiments, which experienced increasing day length and light intensity. It is likely that in spring, earlier anthocyanin production was induced by light rather than cold or osmotic stress. Furthermore, anthocyanin levels gradually reduced throughout juvenile growth and plants recovered their greenery colour. The hypothesis expressed by Hughes *et al*. (2007) fits nicely as an explanation for the recovery in spring plants.

The later return of anthocyanin accumulation in spring was associated with the onset of reproductive development. The variation of colouration observed between families suggests that they may have adopted different strategies, where some withdraw more nutrients from leaves for use in the ongoing reproductive process.

In the case of GH plants, anthocyanin was observed to accumulate only at reproductive stage – regardless of whether they were grown in autumn or spring. This indicates that the sheltered (semi natural) conditions were not sufficiently stressful to induce a protective anthocyanin production response. Accumulation of anthocyanins at flowering appeared lower in GH plants than LE and HE plants. This might be attributed to the more sheltered environment of GH which offered both lower light intensity and warmer temperatures.

Variation between families was observed under controlled cold LD conditions. At 5ºC LD, most plants exhibited a highly intense purple shade, comparable to those in LE and HE, but some families were notable for their lower level of anthocyanin or a complete lack of purple colour (such as seen in family 9A2). All these families were able to produce anthocyanin under some field conditions, suggesting that the genetic differences between them involved responses to environmental cues rather than lack of anthocyanin biosynthesis genes.

In the 5ºC LD experiment, those families with low or no anthocyanin accumulation showed signs of early senescence. This suggests that anthocyanin may play an anti-aging role upon plant tissues, helping to delay the onset of senescence.

The inflorescence growth

The locally collected *A. thaliana* grew rigid woody inflorescence stems. The thickness of the stem varied with growth conditions. The thickened inflorescence stems were likely to be a result of second xylem development. This secondary growth phase, analogous to the process of wood growth in trees, has been suggested to be dependent on a combination of internal developmental and (external) environmental signals (Matsumoto-Kitano *et al*. 2008; Chaffrey *et al*. 2002).

The inflorescence stems of GH plants provided a clear visual demonstration of secondary xylem development. Although LE, HE and GH plants had life cycles of comparable length within the same season, only plants at GH were observed to grow significantly thickened stems. Stem thickening might therefore be associated with one of the factors that differed between GH and the field sites (i.e. sufficient water supply throughout the growth period). However, similar inflorescence stem thickening was observed in several cold controlled experiments (5ºC, 10ºC and 16ºC LD/ SD) but not in the warm (20ºC to 22ºC) controlled experiments, suggesting that low temperature can contribute to increased stem size.

Several local genotypes also developed auxiliary stems fasciation. This natural fasciation in local wild *A. thaliana* was predominantly observed in plants grown in GH during spring and had not been observed in plants grown under controlled conditions (consistent cold or warm temperatures). One possibility is therefore that fasciation occurred in response to a change in temperature: spring grown plants underwent a period of initially cold conditions followed by a 'heat shock' as the temperature warmed over spring (from daily average 10ºC in March to daily average 18ºC in May). Temperature changes have been reported to influence natural fasciation in cultivated plants (White 1948).

However, the re-occurrence rates of fasciation within certain families in GH suggest the possibility of inheritance as a contributory effect in the expression of this phenotype. These local *A. thaliana* are genetically closely related to each other (as described in Chapter 3). If temperature was the primary causal factor for fasciation in these populations, each individual would be expected to have an equal chance of developing fasciation with its cohorts. This would similarly lead to each of the families having an equal chance of fasciation, dependent on conditions, in each of the growing seasons (2008 and 2009).

As described in Table 8-1, family 5A3, 11A5 and 12A1, have at least one family member developed fasciation in two consecutive spring seasons under GH conditions. If temperature was the primary causal factor for fasciation in these populations, each individual would be expected to have an equal chance of developing fasciation. The results therefore suggest that the genotypes of these families make them more prone to fasciation.

In summary, further physiological and histological studies would be needed to confirm the significance of the morphological and colour changes described. These observations lend some circumstantial evidence towards the roles of particular environmental factors; however, experiments under controlled conditions would be needed to identify the factors or combinations of factors that are involved.

Chapter 9 Genetic architecture for growth rate of local *A. thaliana*

Introduction

Growth is a complex trait, affected by many genes, the environment and by gene x environment interactions. When two genotypes exhibiting differences in a trait such as growth are crossed, a continuous distribution of values for this particular trait is often observed in the segregating population. Such a distribution arises when the naturally occurring differences in a phenotypic trait usually involve several genes with relatively small effects (reviewed by MacKay 2001); traits distributed in this way are called quantitative traits. Quantitative trait loci (QTL) are genomic regions that associate with differences in trait values (reviewed by MacKay 2001).

QTL mapping is used to study the genetic architecture of complex traits by estimating the genome regions involved in the traits, the gene effects distribution and gene actions (reviewed by Holland, 2007). The genetic architecture that determines a quantitative trait can be attributed to a moderate number of genes (or QTL) that contribute relatively large effects upon the trait, or to a large number of genes that have small effects on the phenotype (reviewed by Holland 2007; Erickson 2005).

Growth QTL mapping has long been of interest to evolutionary biologists, as a source of insight into adaptation and evolutionary changes. Many of these studies have looked at aspects of growth, ranging from shoot to root growth and growth in response to different abiotic variables. Numerous QTL that affect weight, length and growth rate have been detected in root growth studies (reviewed by Maloof 2003). One of the examples of QTL in shoot growth was reported by Borevitz *et al*. (2002). They measured hypocotyl growth in a L*er*/ Cvi recombinant inbred line (RIL) population under controlled conditions with different wavelengths of light, and identified 12 QTL that correspond to candidate and unknown genes.

In an investigation of genes controlling biochemical variation for secondary metabolites (plant defence compounds), Kroymann and Mitchell-Olds (2005) showed that the genetic interval carrying the methylthioalkylmalate synthase (*MAM*) gene also carried a QTL that influences *A. thaliana* biomass. They also found that this QTL showed epistasis in the direction of the allelic effect – whether an allele increased or decreased biomass was dependent on the genetic background (either *Col*-0 or L*er*-0).

Relative growth rate (RGR) is a comprehensive quantitative trait that characterizes to a large degree the performance of a plant, and is consequently assumed to be component of fitness; faster growing individuals (higher RGR) seem more likely contribute disproportionately to subsequent generations. This is likely to be particularly important in a monocarpic annual such as *A. thaliana*, where vegetative leaves senesce on flowering and nutrients are transferred to reproductive growth, because the size of the plant at flowering is likely to affect its reproductive success.

RGR represents the combined effect of a wide range of physiological processes and environmental variables and is therefore likely to be dependent on multiple genes. Indeed, El-Lithy *et al*. (2004), found multiple QTLs underlying variation in RGR between Landsberg *erecta* (L*er*) and Shakdara (Sha) RILs. They identified a number QTLs that potentially affect growth at different stages. Some these QTLs were also found to affect chlorophyll fluorescence (i.e., photosynthetic efficiency), specific leaf area (dry weight per unit leaf area – a measure of a plant's investment in its leaves) and leaf initiation speed. They concluded that many of the growth QTLs were associated with different physiological functions, where further fine mapping would be required to pinpoint genes that control these apparent pleiotropic effects.

Association mapping is often performed by scanning the entire genome in order to map QTLs of interest - this is based on the likelihood of a marker, such as single nucleotide polymorphism (SNP) or simple sequence repeat (SSR), being associated with the gene variant affecting the trait of interest (Miles and Wayne 2008). With the advance in molecular techniques and analytical tools, classical QTL analyses have also been

combined with genotyping through microarrays (Miles and Wayne 2008; DeCook *et al*. 2006). A DNA microarray is a glass slide which consists of an array of thousands of spots: each spot contains millions of immobilised probe molecules that can be either cDNA or oligonucleotides. Nucleic acids hybridize to either the cDNA or oligo probes on the chip. Allelic variants can be detected because they reduce hybridisation to the probe molecules. If common SNPs are already known, they can be used as probes, allowing a particular SNP allele to be identified. Examples of microarray analysis, such as those offered by NimbleGen, allow two samples to be labelled with different fluorescent dyes and co-hybridised onto the same chip. Polymorphisms can then be detected by differences in the fluorescence ratio of a probe.

In this final chapter, the focus was to generate a segregating QTL mapping population for growth rate. Substantial RGR variation was identified amongst populations collected from around the Edinburgh area. It was also found that growth rate was affected by season to different degrees between families, suggesting that it reflects local adaptation to season. This raised a number of questions, including how many genes are involved in local variation in growth rate, how these genes act at the biochemical and cellular levels and how they determine responses to different seasonal factors in the environment. The objective of this chapter was to screen the whole genome for the potential location of alleles that influence the trait of growth rate in hybrid between fast and slow growing local *Arabidopsis thaliana*. The ultimate goal for mapping populations and the screen was to identify the number of genes responsible for differences between the two selected local *A. thaliana* genotypes, and also determine the position and effect of these genes by QTL analysis.

Results

Growth rate QTL mapping population

Plants to be used as parents for QTL analysis were selected on their previous performance under controlled conditions (20ºC LD) - 4D1 (fast) and 11C1 (slow). The parents were crosses and 87 F2 plants were grown. Seeds were collected from each plant individually after self-pollination and up to six F3 progeny grown from each F2 parent, giving a total mapping population of 507 plants. The mapping population, as well as the parents were grown in a controlled greenhouse with a constant 16 hours light and 8 hours dark at 20ºC. Growth was monitored regularly and the rosettes were photographed on 15 dsg, 20 dsg and 27 for growth estimation. Relative growth rate between 15 dsg and 20 dsg was calculated.

The RGR of individual plants was found to be normally distributed, with a mean value of 0.268 $cm^{-2}cm^{-2}•day^{-1}$. A transgressive segregation for growth rate was observed (Fig. 9-1), suggesting that both parents carried alleles that increased and decreased growth rate The variance within and between F3 families were 0.000731 and 0.007, respectively. This gives an estimate for the heritability, H^2, of RGR of 0.78 - i.e. 78% of the variation in growth observed in the experiment could be attributed to genetic differences. This, however, makes the assumption that members of each F3 family were genetically uniform. Because half of the F2 plants would have been heterozygous for any growth rate QTL, half of the F3 families would show genetically-determined variation due to segregation of that QTL, leading to an underestimate of genetic variance. Thus 0.90 is the minimum value for the heritability of RGR

To identify chromosome regions associated with differences in growth rate, bulk segregant analysis was used. The 12% fastest growing plants (63 individuals from 28 families), as well as the slowest 12% of F3 plants (61 individuals from 36 families), were selected (Fig. 9-1).

At least five of the six plants from the families 040, 045, 046 and 066, were included in the fastest twelfth percentile. This suggests that the parents of these families may have been homozygous for fast alleles of the growth rate QTL, assuming that the alleles acted additively. Of the slowest twelfth percentile, no family, except 075, had greater than four representatives. This suggests that the parents of these individuals could have been heterozygous at growth rate QTL or that slow growth could have a lower heritability than fast. One family, 034, had individuals appearing in both the slow and fast growing categories suggesting that it was heterozygous for growth rate QTL that segregated in its progeny.

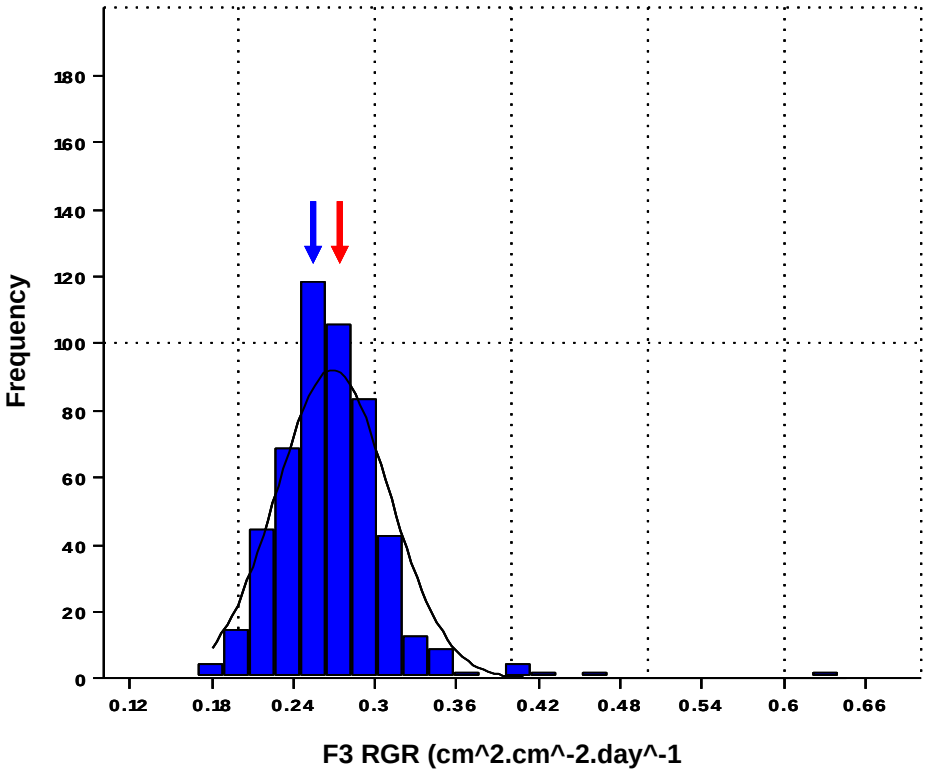

Figure 9-1 **Frequency distribution of relative growth rate in F3 progeny**. Relative growth rate was estimated between 15 and 20 dsg at 20°C under LD conditions. The distribution is approximately normal. The upper and lower twelfth percentiles were selected for bulk segregant analysis. Blue and red arrows denote slow and fast growing parents respectively.

DNA microarray hybridization

DNA was extracted from the selected fast and slow growing plants individually. DNA concentrations were estimated and the same amount of DNA from each plant mixed to make one fast pool and one slow pool. These pools were expected to be enriched for either fast or slow alleles of growth rate QTL. The pooled samples were sonicated into smaller fragments (200–600 bp) for the purpose of hybridizing to the oligo probes (Fig. 9-2A). These pooled DNA fragments were labelled at NimbleGen with the fluorescent dye Cy5 (635 nm emission) for slow individuals and Cy3 (532 nm emission) for fast individuals and then co-hybridized to a microarray consisting of oligonucleotides representing the promoter regions of all predicted genes in the TAIR 9.0 genome annotation (NimbleGen *A. thaliana* Minimal Promoter array). NimbleGen was selected for the array hybridisation because it offered co-hybridization on a single chip. This could minimise errors due to variation between chips and also reduces costs - two colour arrays require half the number of chips.

Different levels of hybridisation of alleles from the parents are expected if polymorphisms exist within the sequences represented by microarray probes. Therefore, polymorphisms that are linked to growth rate QTL are expected to give different levels of hybridization for fast compared to slow pools, whereas alleles of unlinked genes are expected to occur at similar levels in both pools.

The Cy3 and Cy5 emissions of hybridized DNA fragments were measured and returned as raw data by Nimblegen (Fig. 9-2B). These data were processed with ChIPMunk software (http://www.bioinformatics.bbsrc.ac.uk/projects/chipmonk/). Because dyes do not have a linear relationship between intensity and concentration, Lowess normalization was performed to correct dye bias at low intensities and give a more accurate estimate.

Filtering was then performed on the normalised intensity data to identify probes showing distinct differences in Cy3/Cy5 signal ratios. Figure 9-2C is an example of filtered results in which probes with a $\log_2$ ratio of hybridisation signals greater than 1.4 or less

than -1.4 were selected. The probes that survived filtering were frequent and not all clustered in particular chromosome regions, which suggested a high level of background variation. Hybridisation with DNA from each of the parent lines might have helped to identify probes that showed high background differences. However this would have doubled the cost of the experiment.

Given the background of significantly biased signal ratios, attempts were made to identify chromosome regions that were not distributed equally between fast and slow pools, because polymorphisms linked to growth QTL were expected to cluster on chromosomes. The difference in hybridisation signal between pools was expected to be highest around the QTL, as less closely linked polymorphisms are more likely to be separated from the QTL alleles by recombination. Chromosomes were therefore divided into bins and the number of biased probes per bin was plotted. Following tests of different bin sizes, it was determined that a size of 2 mbp (million base pairs) was the most efficient in showing sufficient detail without being too large to observe fine-grained clusters.

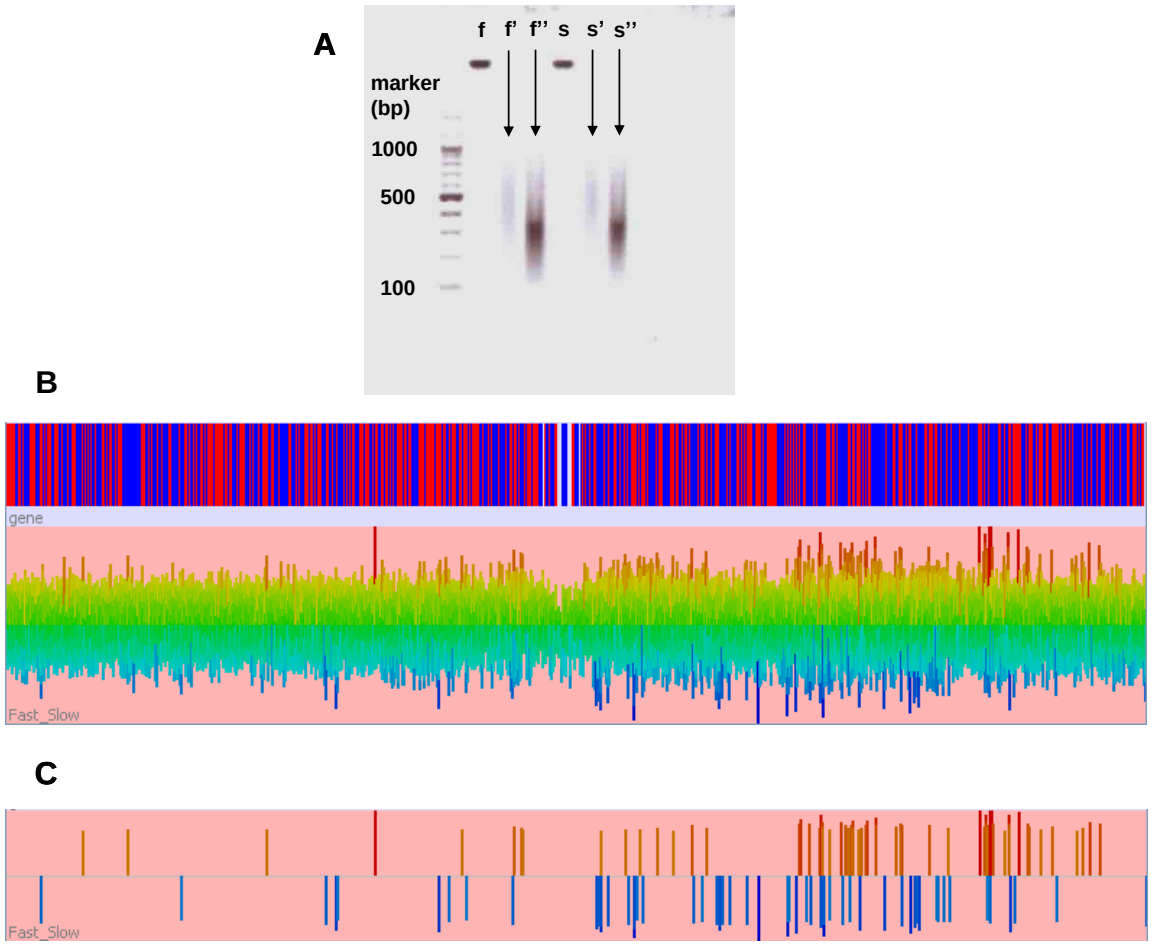

Figure 9-2 **Bulked segregant analysis by microarray hybridization** (**A**) sonicated DNA fragments used for hybridisation. **f** or **s** = fast or slow pooled DNA before sonication, **f'** or **s'** = fast or slow pooled DNA fragments after 90 seconds of sonication, **f''** or **s''** = fast or slow pooled DNA fragments after of 180 seconds sonication;. (**B**) An example of Nimblegen data, for chromosome 1. The distribution of genes (red coding regions, blue promoters) is shown at the top. Unfiltered normalized $\log_2$ probe signal ratios are shown below. Bars above the central line ($\log_2 = 0$) represent probes that gave a greater hybridisation signal with DNA from fast pools relative to slow, those below the line gave a higher hybridisation signal with slow DNA. Bars are coloured according to their values. (**C**) Shows the same data after removal of probes that had a $\log_2$ ratio between -1.4 and 1.4.

DNA hybridization data interpretation

DNA hybridization and analysis identified seven regions in the *A. thaliana* genome that appeared to show biased microarray hybridization. Four out of these seven regions

showed distinct peaks, 16,000,000-28,000,000 bp on chromosome 1; 8,000,000-12,000,000 bp on chromosome 3; and 0-2,000,000 bp and 6,000,000-8,000,000 bp on chromosome 4 (Fig. 9-3Ai – Ei). These regions represent potential locations for growth rate QTL.

However, the density of probes along each chromosome is proportional to gene density. Therefore selecting regions for a high probe density of biased markers might select regions with high gene density rather than growth rate QTL. To remove any effect of gene density, the number of biased probes in each bin was divided by gene density in that bin. Gene density was calculated from the TAIR 7 genome annotation using a Perl script (written by K. Sujai, The Gene Pool, Edinburgh School of Biological Sciences). This corrected data is summarized in Figure 9-3Aii – Eii. One of the highest biases, for example, can be found in the lower arms of Chromosome 1 and 5, where probe frequencies were particularly high. The overall outcome, however, generally supported the previous results (Fig. 9-3Ai – Ei), except the centromeric regions on all chromosomes other than Chromosome 4, showed increased values following correction.

Centromeric regions are known to contain tandem repeat sequences with low gene density in both plant and animal systems. These regions have also been found to exhibit high levels of polymorphism, although the exact cause is unknown. It has been suggested to be either due to a higher mutation rate or bias in the detection of polymorphisms in array-based re-sequencing (Clark *et al*. 2007). The lower values shown on the centromere region of chromosome 4 suggest a less polymorphic centromere.

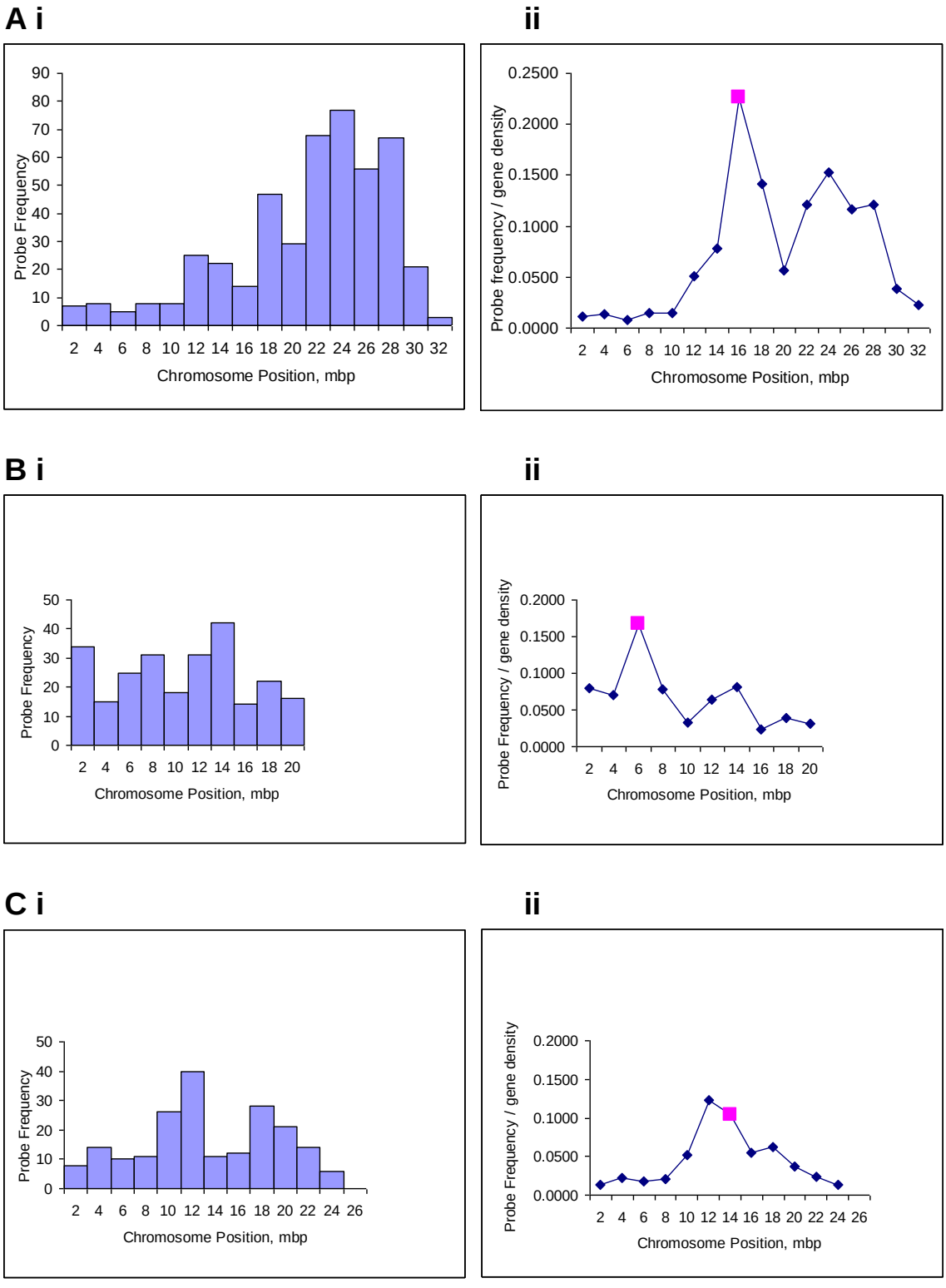

Figure 9-3 **Summary of probes showing biased signal intensity in the *A. thaliana* genome (1 of 2)**.

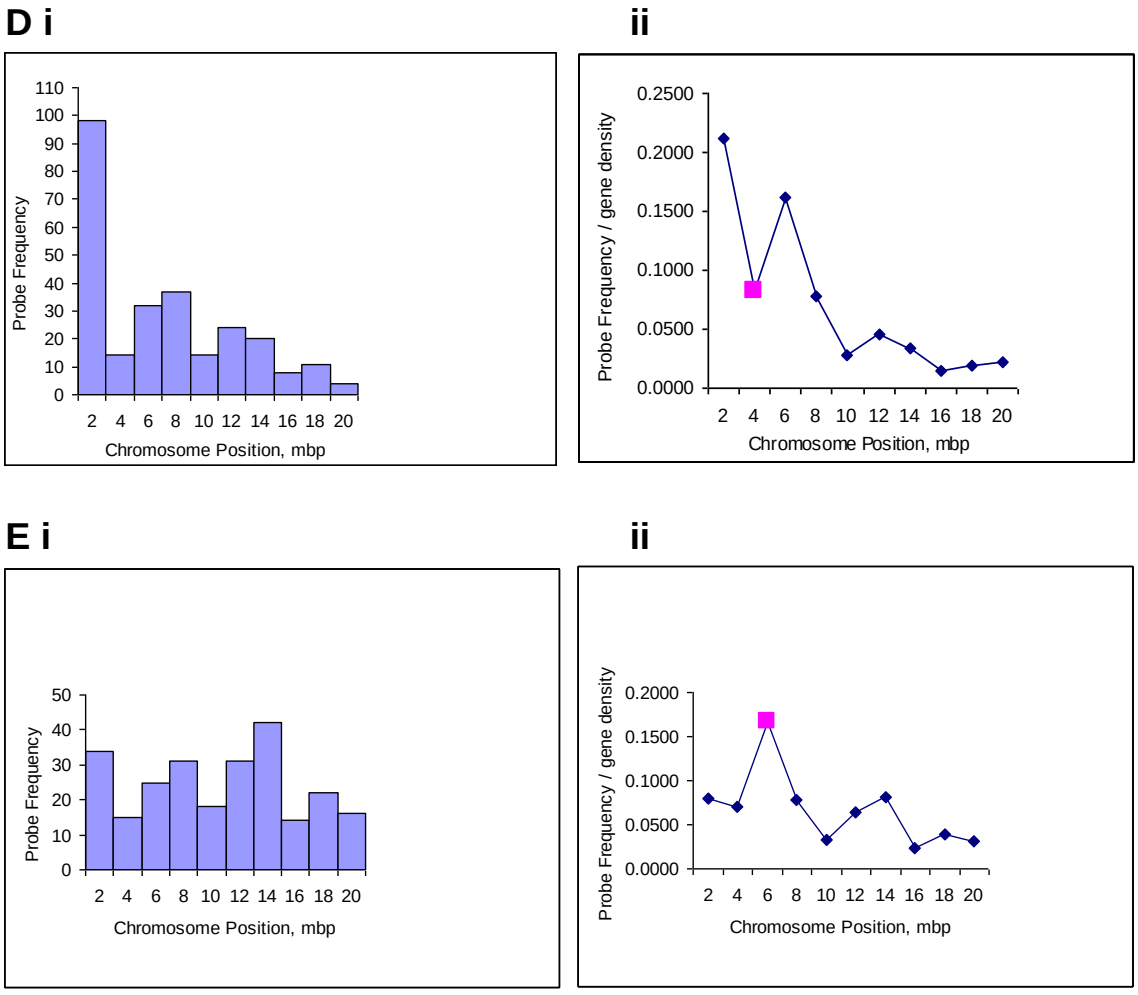

Figure 9-3 **Summary of probes showing biased signal intensity in the *A. thaliana* genome (2 of 2)**. The frequency of biased probes for chromosomes 1-5 is shown in bins of 2 mbp (**A i** – **E i**). The frequency of biased probes per 2 mbp bin after correction for gene density in the bin is shown in **A ii** – **E ii**. The pink dots indicate the approximate locations of centromeres.

Simple sequence length polymorphism (SSLP)

To further test linkage of chromosome regions identified in microarray hybridisation to growth rate QTL, a set of simple sequence length polymorphism (SSLP) markers identified in other *A. thaliana* lab accessions were selected for the candidate regions. SSLPs are excellent markers because they can be easily genotyped and are often polymorphic between different accessions. Single Nucleotide Polymorphism (SNP)

markers were not considered for this study, as SNP alleles are often rare, and therefore less likely to be polymorphic between a pair of accessions and can be more costly to genotype (Agrafioti and Stumpf 2007)

Table 9-1 shows a list of markers with primers sequence available on TAIR. Nine out of the 17 primer pairs that were tested detected polymorphisms. Examples of these polymorphic bands were shown in Figure 9-4.

The primers were used to genotype individuals from the fast pool (the fastest and intermediate fast individuals, consist of plants from six different families), and the slow pool (the slowest and intermediate slow individuals from thirteen different families; Fig. 9-4).

SSLP Markers	Chromosome	AGI (bp)	Polymorphic
CIW1	**1**	**18367549-18367707**	Yes
NGA128	**1**	**20633251-20633430**	Yes
NGA280	**1**	**20877364-20877468**	No
GENEA	**1**	**22400757-22400959**	No
F11P17-4615	**1**	**22601517-22601726**	Yes
F19K23-438	**1**	**22942496-22942696**	No
F5I14-49495	**1**	**24374008-24374203**	Yes
NGA111	**1**	**27356874-27357001**	No
ATPASE	**1**	**28537498-28537582**	No
NGA692	**1**	**28841446-28841564**	No
CIW3	**2**	**6409928-6410163**	Yes
CIW11	**3**	**9775545-9775723**	Yes
CIW4	**3**	**18901818-18902006**	No
CIW6	**4**	**7892620-7892781**	Yes
CIW7	**4**	**11524362-11524492**	Yes
CIW9	**5**	**17061229-17061394**	Yes
NGA1126	**2**	**RI: 50.65-50.65 cM**	No

Table 9-1 **SSLP markers for chromosomal regions enriched in DNA microarray analysis**. The name of the maker, chromosome number, and physical positions are shown. Some tested markers were polymorphic, whereas some were not.

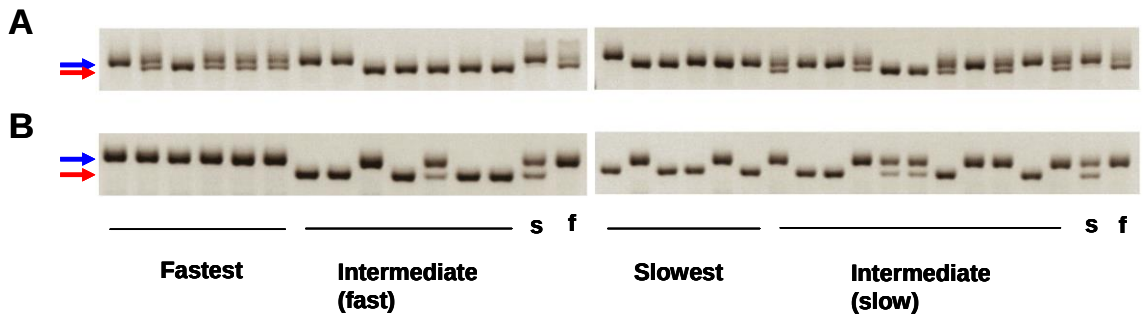

Figure 9-4 **Examples of SSLP analysis**. PCR products of *CIW11* from chromosome 3 **(A)** and *CIW9* from chromosome 5 **(B**) are shown. The product length of *CIW11* is between 179 – 230 bp and *CIW9* is between 145 – 165 bp. The fastest and slowest growing individuals from the QTL mapping population are labelled with **Fastest** and **Slowest**. The individuals with a moderate growth rate (RGR faster or slower than average, but slower than the fastest and vice versa) are labelled **Intermediate**. (**f** = fast pooled DNA and **s** = slow pooled DNA). Blue and red arrows denote bands from slow and fast growing parents respectively.

A Chi-squared test was used in to examine whether the alleles of the SSLP loci were randomly distributed between fast and slow individuals. When segregating mapping population was considered as a single pool, the statistical analysis shows that the distribution of alleles for some markers was biased between fast and slow homozygote (Table 9-2). SSLP markers with significant Chi-squared values ($p < 0.05$), suggest linkage to growth rate QTL. However, individuals in a F3 population are more related to each other if they share an F2 parent, than F2 individuals are to each other. Hence, F3 individuals are more likely to share alleles with their siblings by chance, regardless of whether the alleles are linked to growth rate genes.

The frequencies of the alleles were reconsidered in association with growth rate (Table 9-3). In this case, SSLP alleles are distributed at random between fast and slow-growing individuals. This can be interpreted as indicating that none of the SSLP markers tested are linked to growth rate associated QTL.

SSLP	Observed			Expected			χ^2	*df*	*p*
	GG	Gg	gg	GG	Gg	gg			
CIW1	14	6	22	15.75	10.5	15.75	4.603	2	0.1
NGA128	12	0	4	9.75	6.5	9.75	8.872	2	0.01
F11P17-4615	12	2	44	21.75	14.5	21.75	37.91	2	0.0001
F5I14-49495	14	22	22	21.75	14.5	21.75	6.644	2	0.04
CIW3	12	0	30	15.75	10.5	15.75	24.29	2	0.0001
CIW11	30	18	20	25.5	17	25.5	2.039	2	0.36
CIW6	14	12	14	15	10	15	0.533	2	0.77
CIW7	16	10	14	15	10	15	0.133	2	0.93
CIW9	26	10	32	25.5	17	25.5	4.549	2	0.1

Table 9-2 **Segregation of SSLP alleles in the mapping population**. The probability, *p*, shows that the SSLP alleles are distributed at random in the sampled plants. Key: **GG** = homozygous for the allele from the fast growing parent; **gg** = homozygous for the allele from the slow growing parent; **Gg** = heterozygous.

SSLP	Observed				Expected		χ^2		df	*p*	
	Fast Pool		Slow Pool		Fast	Slow	Fast	Slow		Fast	Slow
	G	g	G	g							
CIW1	8	8	9	15	8	12	0	1.5	1	1.0	0.22
NGA128	6	6	8	4	6	6	0	1.333	1	1.0	0.25
F11P17-4615	7	19	6	26	13	16	5.538	12.5	1	0.019	0.0004
F5I14-49495	11	15	14	18	13	16	0.615	0.5	1	0.43	0.48
CIW3	14	2	14	10	8	12	9.0	0.667	1	0.0027	0.41
CIW11	10	16	25	9	13	17	1.385	7.529	1	0.24	0.006
CIW6	7	13	13	7	10	10	1.8	1.8	1	0.18	0.18
CIW7	8	12	13	7	10	10	0.8	1.8	1	0.37	0.18
CIW9	11	15	16	16	13	16	0.615	0	1	0.43	1.0

Table 9-3 **Association of SSLP alleles with growth rate**. The probability, *p*, shows that the SSLP alleles are distributed at random between fast and slow-growing individuals. In this case, the frequencies of the alleles were considered separately. Key: **G** = the allele from the fast growing parent; **g** allele from the slow growing parent.

Discussion

Most complex traits, i.e. growth rate, are usually conditioned by more than one locus.

Growth rate associated QTLs often interact in complex ways and their expression can

also be influenced by environmental factors. Importantly, in this chapter RGR was found to have a substantial H^2, even though it has been previously observed that RGR could be strongly affected by the environment (Chapter 7). This suggests that the environmental variation between plants was not large. This also indicates that the segregating population offers promise for mapping growth rate QTL because the power of QTL detection is directly related to the strength of H^2 (Utz and Melchinger 1994; reviewed by Tanksley 1993)

The QTL mapping population presented in this chapter was shown to segregate for growth rate. In addition, use of DNA microarray co-hybridisation for genome-wide growth rate QTL screening was shown to be suitable for F3 mapping population.

In the segregating mapping population, the pool of fast plants were expected to be enriched for QTL alleles that increase growth rate and therefore also enriched for polymorphisms linked to these alleles, with the level of enrichment depending on the degree of linkage. Similarly, slow growing plants would be enriched for polymorphisms linked to QTL alleles that decrease growth rate. Indeed, over half of the SSLP markers selected from around the enriched regions as indicated by the microarray were highly polymorphic. However, most of the marker alleles themselves appeared to randomly distributed, indicating very low to no linkage to growth rate associated QTL.

There are several issues that could lead to the observed results. Firstly, there was no control for differential hybridisation of parental sequences to the microarray probes; therefore it is not clear whether chromosome regions which showed differential expression to DNA from fast and slow pools reflected enrichment for sequences linked to growth rate QTL. Secondly, the F3 plants were derived from relatively few F2 parents; therefore members of the mapping population with the same F2 parent will share many alleles in common by chance. This will reduce the ability to detect QTL, particularly if they are affected by genetic background (epistasis). Thirdly, although growth rate appears highly heritable, it might involve a relatively large number of loci with small effects.

Another possible issue - is that not all of the microsatellites were polymorphic between the mapping parents, as the percentage of loci that successfully amplify may decrease with increasing genetic distance (Jarne and Lagoda 1996). It is possible that the monomorphic markers could have been more closely linked to QTLs.

There are potential effects of heterosis; where hybrids display superior traits over their parents. Several families from the mapping population (i.e. 040 and 045) exhibited a more vigorous RGR than the fast parent. For example, the RGR for family 040 was 0.42 ± 0.04 cm^2cm^{-2}•day^{-1} (mean ± SE, n = 6) compared to 0.27 ± 0.004 cm^2cm^{-2}•day^{-1}, for the faster parent. This might occur because both parents carry alleles that increase and decrease growth rate, so that hybrids can inherit more increasing or decreasing alleles than their parents. However, it might also reflect heterosis (reviewed by Lippman and Zamir 2007; Tanksley 1993). If heterosis is important then the bulked-segregant approach would not be able to detect loci involved in heterosis, because fast growing plants would be heterozygous and slow growing a mixture of the two homozygotes – the ratio of parental alleles would be similar in both cases.

In summary, the genome-wide microarray screening identified the potential chromosomal locations of growth rate QTL. However, proceeding from the initially microarray to map growth rate QTL remains. The next step could involve using a larger population (i.e., more recombinant chromosomes), along with a higher density of molecular markers for linkage detection and to infer the position of the QTL. The number of markers tested is probably not sufficiently dense to achieve the potential QTL linkage detection - sparse marker maps severely limit the power of QTL mapping (reviewed by Doerge 2002). The likelihood of linkage could then be accessed by test statistics. Ultimately, the stability and effects of QTL will be tested and estimated, across different environment and within different genetic backgrounds.

Chapter 10 Summary and Conclusion

Understanding *A. thaliana* in its native context provides insights into how plants are adapted to their environments and how populations will respond to changing environments. In particular, ongoing climate change will require a deep understanding of how both wild and cultivated plant species respond. As *A. thaliana* is a model organism, the insights gained in this work can be applied to other plant species – including crops – and are intended to compliment an increasing body of recent research into natural variation in wild *A. thaliana*.

Local populations of *A. thaliana* were found to be highly polymorphic, consistent with previous studies on global accessions. Genome-wide DNA fingerprinting elucidated the relationships within and among local *A. thaliana* populations. As suggested by the AFLP data, most of the local individuals used in this study did not appear to be recent migrants, but to have been sufficiently well established to give rise to a substantial range of genetic variation, either through mutation or recombination, so that individuals from the same site tended to be genetically most similar to each other, but not identical. *Arabidopsis thaliana* – a globally distributed selfing species with a low outcrossing rate - has been found to be highly polymorphic with low linkage disequilibrium (Nordborg *et al.* 2002). Low linkage disequilibrium implies that the original allele has been broken down by recombination following cross-pollination between genetically different parents (Nordborg *et al.* 2005).

An investigation of growth under natural conditions was performed using a common garden approach. If growth rate is adaptive, the variation was expected to be heritable in field conditions. This proved to be the case. It was also expected to affect seed yield, as it did in some conditions.

The accessions had been collected from sites ranging in elevations from 62 m to 249 m, therefore one hypothesis was that they would be locally adapted to environmental factors

that varied with elevation (e.g. temperature). Genotypes differed in their growth rate in the field at two extreme elevations (which differed, on average, by 2°C). However, the growth rate of the accessions at the two different sites was strongly correlated – i.e., there was no evidence that genotypes from higher elevations were adapted to higher elevations, and *vice versa*.

However, some genotypes exhibited better growth performance in the autumn-winter period than spring, or *vice versa*, suggesting that they might be adapted to either a winter annual or summer annual lifestyle.

A subset of local *A. thaliana* accessions that showed different seasonal fitness responses were grown under controlled chambers, simulating each of the seasonal conditions that plants experienced in the field separately. These controlled experiments support the view that environmental factors –temperature and daylength - have a combinatorial effect upon growth rates.

Summer annuals have traditionally been seen as rapid cycling genotypes that are not responsive to vernalization (e.g. *fri* mutants). It was initially expected that, as is typical of Northern European accessions, *A. thaliana* from the Edinburgh region would be biennial (also referred as winter annuals); requiring vernalization in line with the seasonal climate changes of a temperate region. These local accessions were collected as rosettes between the end of winter and beginning of spring, supporting this expectation – as summer annuals would be expected to germinate in spring rather than reach rosette stage.

Evidence presented in this thesis shows that all genotypes completed their life-cycle as summer annuals (with the exception of the latest flowering accessions under conditions of dry springs); germinating in spring and fruiting by summer. Similarly, few genotypes flowered in the autumn before the onset of winter and therefore no clear distinction could be drawn between rapid cycling summer annuals and winter annual lifestyles.

Differences in flowering time expression were observed in the autumn and spring experiments (Chapter 5-2). Genetic variation had less impact on flowering time in autumn germinated plants; the growth of all plants slowed down to halt in winter and resumed in time to flower almost simultaneously in spring. Summer, however, showed a greater range of flowering times. Flowering time variation in the summer was also observed to have a greater association with fitness; earlier flowering genotypes grew more seed, whereas late flowering genotypes did not always make it to fruit production (Chapter 5-3). Therefore if flowering time is adaptive, it is more likely to be selected for delaying flowering with prolonged vegetative growth in the spring, than for preventing flowering in autumn.

Timing of germination potentially influences the evolution of all traits expressed during the lifecycle of an individual. Dormancy allows for what may be described as a 'bet hedging' strategy, where the environment above ground is unpredictable and inhospitable; germination cues allow growth to begin under more optimal conditions (reviewed by Donohue *et al*. 2010). Temperature is regarded as the key environmental factor for the regulation of dormancy in temperate regions (Baskin and Baskin 1988). For example, high summer temperatures tend to impose dormancy whereas cold winter temperatures break it (reviewed by Donohue *et al*. 2010; Bouwmeester and Karssen 1993; Baskin and Baskin 1988). It is probable that seed dormancy plays an important role in determining whether plants grow as biennials or summer annuals.

Variation in seed dormancy was observed in local accessions. Some genotypes were observed to be highly responsive to vernalization, whereas some were not. One area of interest for future research would be to determine whether a correlation exists between the observed variation in seed dormancy and growth performance of genotypes in autumn-winter and spring, to determine whether dormancy is an adaptive response to local climate conditions.

In addition, the lack of strong dormancy (a trait which can be highly genetically determined) in some genotypes would allow seedlings to germinate and grow two

generations per year, i.e. germination in spring and then autumn. This rapid growth cycle can be facilitated by the local weather conditions in Edinburgh – wet all year round with relatively mild winter and cool summer. This could potentially lead to a mixture of two genetically differing populations germinating in the same season, i.e. autumn; one which has strong dormancy that germinate after vernalization, and another which lacks strong dormancy and opportunistically takes advantage of conditions suitable for germination. One potentially interesting avenue of research would be investigate the demographics of local accessions and population structure, in order to determine whether the local population is purely strongly dormant winter annuals or a mixture of different types.

The United Nations Intergovernmental Panel on Climate Change (IPCC) has estimated (4th Assessment Report, 2007) a potential global temperature rise of 4.0 °C (in the likely range 2.4-6.4 °C) over the next century. The seasonal temperature variation in Edinburgh from 2007 to 2010 is greater than this value; as recorded in chapter 4, for example, the minimum annual temperature is 5°C and the maximum 16°C. If local populations are able to switch between being winter and summer annual behaviour, this offers the potential for adaptation to climate change (specifically, to increased temperatures) as summer annuals can adapt to function as winter annuals growing in now-warmer winter temperature. Evidence for such genetic variation can already be found in the observed ability of these populations to change from typical biennial behaviour (at the time of collection) to summer annual (as seen during the field growth experiments).

In the investigation of competition involvement in adaptation, it is found that the identity of the neighbour could affect both growth rate and fitness of *A. thaliana* in competition. However, the outcome of competition could be broadly predicted from the performance of genotypes in isolation.

To understand the genetic basis of growth rate, an experiment was set up to identify growth rate associated QTL. Fast growing and slow growing plants, as determined under controlled warm and long day conditions, were selected and crossed. The segregating F3 progeny were pooled for DNA microarray hybridization. Four chromosomal regions

showing signal enhancement were detected; these regions represent potential growth-rate associated QTL. The involvement of multiple QTL is consistent with the observation that the growth rates of genotypes respond differently to day length and to temperature, suggesting that seasonal adaptation can be achieved in different ways genetically.

There were several complicating factors when conducting growth experiments in the field. Firstly, although seasonal weather patterns were generally consistent, there remained variation within the season itself. For example, factors such as precipitation and temperature could fluctuate unpredictably within the season – even whilst the overall trends remained similar year on year. In order to more fully account for such variation, further field experiments would be required across a very long timescale; unfortunately, time constraints restricted the viable study time for this work.

Secondly, herbivore predation had an impact on plant growth; some genotypes were observed to be more susceptible to slug predation than others during seasonal common garden experiments. This observation suggested a possibility that local genotypes have different levels of glucosinolates (mustard oil glucosides); nitrogen- and sulfur-containing natural plant products known to provide effective defence against generalist herbivores and pathogens. *A. thaliana* has long been known to contain aliphatic, aromatic and indole glucosinolates (reviewed by Wittstock and Halkier 2002). The level and types of glucosinolates vary extensively across the different global accessions (Kliebenstein *et al*. 2001). Methylthioalkylmalate synthase1, *MAM*1 for example, is a main gene controlling glucosinolate side-chain length (reviewed by Wittstock and Halkier 2002). It has been previously shown by Kroymann and Mitchell-Olds (2005) that the genetic interval carrying the methylthioalkylmalate synthase (*MAM*) gene also carried a QTL that influences *A. thaliana* biomass. Therefore, it was of interest to study predator resistance of local genotypes – to account for the possible utilization of resources to deter predators, at the expense of immediate growth and associated fitness costs.

A preliminary study was conducted (with assistance in slug species identification from G. Port of the University of Newcastle), to examine slug predation. This involved

examining foraging behaviour and dietary preference of local slugs. Captive slugs were placed within a constrained environment containing potential food. Selected local *A. thaliana* genotypes from common garden experiments (based on those observed to be susceptible to, or unaffected by, predation) were placed alongside cabbage (a fellow member of the Brassicaceae family) or lettuce (a member of Asteraceae). The visibility of food within the environment was varied through covering or uncovering.

The work looked to investigate whether susceptibility to predation could be attributed to slug foraging behavior in identifying and targeting the individual plants as preferable food; for example, where plants possessed different levels of glucosinolates. It was found that slugs preferred lettuce over cabbage, and both over *A. thaliana*. Also, slugs took longer to forage when alternate food was covered, but still avoided *A. thaliana* in favour of the other food types. These preliminary results did not indicate any slug preference for or against particular *A. thaliana* genotypes. However, it is clear that *A. thaliana* has a stronger repulsive effect, presumably due to higher deterrent chemicals content compared to the other two food choices. A more definitive conclusion would require larger scale study with an increased number of slug species and individual slugs.

In conclusion, this thesis has presented evidence that the diverse phenotypic characteristics of local wild *Arabidopsis thaliana* are adaptive - growth rate in particular was found to be highly heritable under both natural and controlled conditions. The investigation of growth rate variation has also suggested seasonal adapted growth, which has not been previously reported. It was found that both growth rate and flowering time could be adaptive characters which have an important role in determining overall fitness. For example, those genotypes exhibiting fast growth in autumn and flowering earlier in spring (before the onset of short summer drought) grew more seeds than slower growing, late flowering plants.

References

Abbot RJ and Gomes MF: Population Genetic Structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity. 1989; 62:411-418.

Abe K, Osakabe K, Ishikawa Y, Tagiri A, Yamanouchi H, Takyuu T, Yoshioka T, Ito T, Kobayashi M, Shinozaki K, Ichikawa H and Toki S: Inefficient double-strand DNA break repair is associated with increased fasciation in Arabidopsis *BRCA2* mutants. JXB. 2009; 60 (9): 2751–276. (doi:10.1093/jxb/erp135)

Abecasis GR, Ghosh D and Nichols TE: Linkage Disequilibrium: Ancient History Drives the New Genetics. Human Heredity. 2005; 59:118-124

Adobe Systems Incorporated; Adobe Photoshop 7.0.

Agrafioti I and Stumpf MPH: SNPSTR: A database of compound microsatellite-SNP markers. Nucleic Acids Research. 2007; 35: D71-D75. (doi: 10.1093/nar/gkl806)

Ågren J, Schemske DW: Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. New Phytologist. 2012; 194: 1112–1122. (doi: 10.1111/j.1469-8137.2012.04112.x)

Aitken SN, Yeaman S, Holliday JA, Wang TL and Curtis-McLane S: Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol. Appl. 2008; 1: 95–111. (doi:10.1111/j.1752-4571.2007.00013.x)

Allendorf FW, Luitken GH and Aitken SN: Conservation and the genetics of populations. Wiley-Blackwell, 2nd edition. 2013.

Alonso-Blanco C, Blankestun-De Vries H, Hanhart CJ and Koornneef M: Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. PNAS. 1999; 96: 4710–4717.

Alonso-Blanco C, Mendez-Vigo B and Koornneef M: From phenotypic to molecular polymorphisms involved in naturally variation of plant development. Int. J. Dev. Biol. 2005; 49:717-737.

Alonso-Blanco C, Aarts MGM, Bentsinkc L, Keurentjes JJB, Reymond M, Vreugdenhild D and Koornneef M: What Has Natural Variation Taught Us about Plant Development, Physiology, and Adaptation. The Plant Cell. 2009; 21: 1877-1896. (doi:10.1105/tpc.109.068114)

Al-Shehbaz IA and O'Kane Jr SL: Taxonomy and Phylogeny of Arabidopsis (Brassicaceae). The Arabidopsis Book. 2002; doi/10.119/tab.001.

Amasino R: Seasonal and developmental timing of flowering. The Plant Journal. 2010; 61: 1001–1013. (doi:10.1111/j.1365-313X.2010.04148.x)

Arany AM, de Jong TJ and van der Meijden E: Herbivory and local genetic differentiation in natural populations of *Arabidopsis thaliana* (Brassicaceae). Plant Ecol. 2009; 201: 651–659. (doi: 10.1007/s11258-008-9530-y)

Atkinson J: Quantitative trait locus analysis of growth in *Arabidopsis thaliana*. PhD Thesis, University of Edinburgh. 2006.

Bakker EG, Stahl EA, Toomajian C, Nordborg M and Kreitman M: Distribution of genetic variation within and amongst local populations of *Arabidopsis thaliana* over its species range. Molecular Ecology. 2006; 15:1405-1418.

Banta JA, Dole J, Cruzan MB and Pigliucci M: Evidence of local adaptation to coarse-grained environmental variation in *Arabidopsis thaliana*. Evolution. 2007; 61:2419–2432. (doi:10.1111/j.1558-5646.2007.00189.x)

Baskin CC and Baskin JM: 1988. Germination ecophysiology of herbaceous plant species in a temperate region. Am. J. Bot. 1988; 75(2): 286-305.

Baskin JM and Baskin CC: Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. Botanical Gazette. 1983; 144 (4): 540–543.

Beck JB, Schmuths H and Schaal BA: Native range genetic variation in *Arabidopsis thaliana* is strongly geographically structured and reflects Pleistocene glacial dynamics. Mol Ecol. 2008; 17: 902-915

Bergelson J, Stahl E, Dudek S and Kreitman M: Genetic variation within and among populations of *Arabidopsis thaliana*. Genetics. 1998; 148: 1311–1323.

Berry J and Björkman O: Photosynthesis response and adaptation to temperature in higher plants. Annu Rev Plant Physiol. 1980; 31: 491-543.

Biedrzycki ML, Jilany TA, Dudley SA and Bais HP: Root exudates mediate kin recognition in plants. Commun Integr Biol. 2010; 3 (1): 28–35. (doi:10.4161/cib.3.1.10118)

Blazquez MA, Ahn JH and Weigel D: A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nature Genetics. 2003; 33: 168-171. (doi:10.1038/ng1085)

Bomblies K, Yant L, Laitinen RA, Kim ST, Hollister JD, Warthmann N, Fitz J and Weigel D: Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. PLoS Genetics. 2010; 6 (3): e1000890. (doi:10.1371/journal.pgen.1000890)

Bonser SP and LW Aarssen: Meristem allocation: A new classification theory for adaptive strategies in herbaceous plants. Oikos. 1996; 77 (2): 347-352.

Bonser SP and Geber MA: Growth form evolution and shifting habitat specialization in annual plants. J. Evol. Biol. 2005; 18:1009–1018. (doi:10.1111/j.1420-9101.2005.00904.x)

Borevitz JO, Maloof JN, Lutes J, Dabi T, Redfern JL, Trainer GT, Werner JD, Asami T, Berry CC, Weigel D, and Chory J: Quantitative trait loci controlling light and hormone response in two accessions of Arabidopsis thaliana. Genetics. 2002; 160 (2): 683–696.

Bouwmeester HJ and Karssen CM 1993: Annual changes in dormancy and germination of *Sisymbrium officinale* (L.) Scop. New Phytologist. 1993; 124: 179-191.

Breyne P, Rombaut D, Van Gysel A, Van Montagu M and Gerats T: AFLP analysis of genetic diversity within and between *Arabidopsis thaliana* ecotypes. Mol Gen Genet. 1999; 261: 627-634.

Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J and Purugganan MD: Epistatic interaction between Arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. PNAS. 2004; 101 (44): 15670-15675 (doi:10.1073/pnas.0406232101)

Cahaner A and Hillel J: Estimating heritability and genetic correlation between traits from generations F_2 and F_3 of self-fertilizing species: a comparison of three methods. Theoretical and Applied Genetics. 1980; 58 (1): 33-38. (doi:10.1007/BF00264666)

Callahan HS and Pigliucci M: Shade-induced plasticity and its ecological significance in wild populations of Arabidopsis thaliana. Ecology. 2002; 83 (7): 1965-1980.

Callaway RM: The detection of neighbors by plants. Trends Ecol. Evol. 2002; 17: 104-105. (doi:10.1016/j.physletb.2003.10.071)

Casper B: Plant competition underground. Annu. Rev. Ecol. Syst. 1997; 28: 545-570. (doi:10.1146/annurev.ecolsys.28.1.545)

Castiglioni P, Ajmone-Marsan P, van Wijk R and Motto M: AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group ditsribution.Theor Appl Genet. 1999; 99 (34): 425-431. (doi:10.1007/s001220051253)

Chaffey N, Cholewa E, Regan S and Sundberg B: Secondary xylem development in *Arabidopsis*: a model for wood formation. Physiologia Plantarum, 2002; 114: 594–600. (doi:10.1034/j.1399-3054.2002.1140413.x)

Chalker-Scott L: Environmental significance of anthocyanins in plant stress responses. Photochemistry and Photobiology. 1999; 70: 1-9 (doi: 10.1111/j.1751-1097.1999.tb01944.x)

Chalker-Scott L: Do anthocyanins function as osmoregulators in leaf tissues. Advance in Botanical Research. 2002; 37: 103-106 (doi:10.1016/S0065-2296(02)37046-0)

Charlesworth D and Vekemans X: How and when did *Arabidopsis thaliana* become highly self-fertilising. BioEssays. 2005; 27(5):472-476

Ciccarelli FD, Doerks T von Mering C, Creevey CJ, Snel B and Bork P: iTOL v1.0. Science. 2006; 311 (5765): 1283-1287.

Cheplick GP: Sibling competition in plants. Journal of Ecology, 1992; 80 (3): 567-575.

Clark RM, Schweikert G, Toomajian C, Ossowski S, Zeller G, Shinn P, Warthmann N, Hu TT, Fu G, Hinds DA, Chen H, Frazer KA, Huson D, Scholkopf B, Nordborg M, Ratsch G, Ecker JR and Weigel D: Common Sequence Polymorphisms Shaping Genetic Diversity in *Arabidopsis thaliana*. Science. 2007; 317: 338-342. (doi:10.1126/science.1138632)

Clark SE, Jacobsen SE, Levin JZ and Meyerowitz: The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in Arabidopsis. Development. 1996; 122:1567-1575.

Clarke SE: Cell signalling at the shoot meristem. Nature Reviews. 2001; 2: 276-284. (doi:10.1038/35067079)

Clausen J, Keck DD and WM Hiesey: Experimental studies on the nature of species III. Environmental responses of climatic race of Achillea. Carnegie Institute of Washington Publication. 1948.

Clayton WD: Evolution and distribution of grasses. Ann Mo Bot Gard. 1981; 68: 5-14.

Cookson SJ and Granier C: A Dynamic Analysis of the Shade-induced Plasticity in Arabidopsis thaliana Rosette Leaf Development Reveals New Components of the Shade-adaptive Response. Annals of Botany 2006; 97 (3): 443-452. doi: 10.1093/aob/mcj047

Criddle, RS, Hansen LD, Smith BN, Macfarlane C, Church JN, Thygerson T, Jovanovic T, and Booth T: Thermodynamic law for adaptation of plants to environmental temperatures. Pure Appl. Chem. 2005; 77 (8): 1425–1444. (doi:10.1351/pac200577081425)

de Kroon H: How do roots interact. Science. 2007; 318: 1562-1563. (doi:10.1126/science.1150726)

de Meaux J and Mitchell-Olds T: Evolution of plant resistance at the molecular level: Ecological context of species interactions. Heredity. 2003; 91: 345-352.

DeCook R, Lall S, Nettleton D and Howell SH: Genetic Regulation of Gene Expression During Shoot Development in Arabidopsis. Genetics. 2006; 172: 1155-1164. (doi:10.1534/genetics.105.042275)

DePuit EJ and Caldwell MM: Seasonal pattern of net photosynthesis of *Artemisia tridentate*. Am J Bot. 1973; 60 (5): 426-435.

Diaz C, Saliba-Colombani V, Loudet O, Belluomo P, Moreau L, Daniel-Verde F, Morot-Guadry JF and Masclaux-Daubresse C: Leaf Yellowing and Anthocyanin Accumulation are Two Genetically Independent Strategies in Response to Nitrogen Limitation in Arabidopsis thaliana. Plant Cell Physiol. 2006; 47 (1): 74-83. (doi:10.1093/pcp/pci225)

Diaz M, De Haro V, Muñoz, R and Quiles M: Chlororespiration is involved in the adaptation of *Brassica* plants to heat and high light intensity. Plant, Cell & Environment. 2007; 30: 1578–1585. (doi:10.1111/j.1365-3040.2007.01735.x)

Doerge RW: Mapping and analysis of quantitative trait loci in experimental populations. Nat. Rev. Genet. 2002; 3: 43–52.

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, and Willis CG: Germination, postgermination adaptation, and species ecological ranges. Annu Rev Ecol Evol Syst. 2010; 41:293-319. (doi:10.1146/annurev-ecolsys-102209-144715)

Donohue K: Completing the cycle: maternal effects as the missing link in plant life histories. Phil Trans R Soc B. 2009; 364: 1059-1074. (doi:10.1098/rstb.2008.0291)

Dudley SA: Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypotheses. Evolution. 1996; 50 (1): 92-102.

Dudley SA and File AL: Kin recognition in an annual plant. Biology Letters. 2007; 3: 435-438. (doi:10.1098/rsbl.2007.0232)

El-Lithy ME, Clerkx EJM, Ruys GJ, Koornneef M and Vreugdenhil D: Quantitative Trait Locus Analysis of Growth-Related Traits in a New Arabidopsis Recombinant Inbred Population. Plant Physiology. 2004; 135:444–458. (doi:10.1104/pp.103.036822)

Erickson D: Mapping the future of QTL's. Heredity. 2005; 95: 417–418. (doi:10.1038/sj.hdy.6800699)

Eriksson S, Böhlenius H, Moritz T and Nilsson O: GA4 is the active gibberellin in the regulation of *LEAFY* transcription and Arabidopsis floral initiation. The Plant Cell. 2006; (doi/10.1105/tpc.106.042317)

Falconer DS: Introduction to quantitative genetics. Longman, London 2nd edition. 1981.

Falconer DS and Mackay TFC: Introduction to quantitative genetics. Longman, New York 4th edition. 1996; 464.

Falik O, Reides P, Gersani M and Novoplansky A: Self/ non-self discrimination in roots. Journal of Ecology. 2003; 91: 525–531. (doi:10.1046/j.1365-2745.2003.00795.x)

Fambrini M, Bonsignori E, Rapparini F, Cionini G, Michelotti V, Bertini D, Baraldi R and Pugliesi C: *stem fasciated*, a recessive mutation in sunflower (*Helianthus annuus*), alters plant morphology and auxin level. Ann Bot. 2006; 98 (4): 715-730. (doi:10.1093/aob/mcl153)

Feild TS, Lee DW and Holbrook NM: Why Leaves Turn Red in Autumn. The Role of Anthocyanins in Senescing Leaves of Red-Osier Dogwood. Plant Physiology. 2001; 127: 566-574. (doi:10.1104/pp.010063)

Fine PV, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi I, Schultz JC and Coley PD: The growth-defense trade-off and habitat specialization by plants in Amazonian forests. Ecology. 2006; 87 (7): S150-S162.

Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J and Wilczek AM: A Map of local adaptation in Arabidopsis thaliana. Science. 2011; 334 (6052): 86-89. (doi:10.1126/science.1209271)

Franklin KA: Shade avoidance. New Phytologist. 2008; 179: 930–944. (doi:10.1111/j.1469-8137.2008.02507.x)

Gersani M, Brown JS, O'Brien EE, Maina GM and Abramsky Z: Tragedy of the commons as a result of root competition. J Ecol. 2001; 89: 660-669. (doi: 10.1046/j.0022-0477.2001.00609.x)

Gillespie JH and Turelli M: Genotype-Environment Interactions and the maintenance of polygenic variation. Genetics. 1989; 121 (1): 129-138.

Griffith M, Timonin M, Wong ACE, Gray GR, Akhter SR, Saldanha M, Rogers MA, Weretilnyk EA and Moffatt B: Thellungiella: an Arabidopsis-related model plant adapted to cold temperatures. Plant, Cell and Environment. 2007; 30: 529–538. (doi:10.1111/j.1365-3040.2007.01653.x)

Gruntman M and Novoplansky A: Physiologically mediated self/ non-self discrimination in roots. PNAS. 2004; 101 (11): 3863-3867. (doi:10.1073/pnas.0306604101)

Hatier J-BB, Gould KS: Anthocyanin function in vegetative organs. Anthocyanins. 2009; 1-19. (doi: 10.1007/978-0-387-77335-3_1)

Hall MC and Willis JH: Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution. 2006; 60(12): 2466–2477. (doi: 10.1111/j.0014-3820.2006.tb01882.x)

Hall MC, Lowry DB and Willis JH: Is local adaptation in Mimulus guttatus caused by trade-offs at individual loci. Molecular Ecology. 2010; 19: 2739-2753. (doi:10.1111/j.1365-294X.2010.04680.x)

Hammer O and Harper DAT, PAST v1.83. http://folk.uio.no/ohammer/past/

Hancock AM, Brachi B, Faure N, Horton MW, Jarymowycz LB, Sperone FG, Toomajian C, Roux F and Bergelson J: Adaptation to climate across the Arabidopsis thaliana genome. Science. 2011; 334 (6052): 83-86. (doi:10.1126/science.1209244)

Harper JL, Lovell PH and Moore KG: The shapes and sizes of seeds. Annu. Rev. Ecol. Syst. 1970; 1: 327-356 (doi: 10.1146/annurev.es.01.110170.001551)

Hedrick PW: Genetic polymorphism in heterogeneous environments: The age of genomics. Annu. Rev. Ecol. Evol. Syst. 2006; 37: 67-93. (doi:10.1146/annurev.ecolsys.37.091305.110132)

Heisey RM: Allelopathic and herbicidal effects of extracts from tree of heaven (*Ailanthus altissima*). American Journal of Botany. 1990; 77 (5): 662-670.

Hoch WA, Singsaas EL, and McCown BH: Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiology. 2003; 133: 1296-1305. (doi:10.1104/pp.103.027631)

Hoffmann AA and Sgrò CM: Climate change and evolutionary adaptation. Nature. 2011; 470: 479-485. (doi:10.1038/nature09670)

Hoffmann MH: Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). J. Biogeography. 2002; 29: 125-34.

Hoffmann WA and Poorter H: Avoiding bias in calculations of relative growth rate. Annals of Botany. 2002, 80: 37-42. (doi:10.1093/aob/mcf140)

Holland JB: Genetic architecture of complex traits in plants. Current Opinion in Plant Biology. 2007; 10 (2): 156-161. (doi:10.1016/j.pbi.2007.01.003)

Holmes MG and Smith H: The function of phytochrome in the natural environment. II. The influence of vegetation canopies on the spectral energy distribution of natural daylight. Photochemistry and Photobiology. 1977; 25: 539–545. (doi:10.1111/j.1751-1097.1977.tb09125.x)

Hughes NM, Morley CB and Smith WK: Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. New Phytologist. 2007; 175: 675–685. (doi: 10.1111/j.1469-8137.2007.02133.x)

Hus H: Fasciation in Oxalis crenata and Experimental Production of Fasciations. Missouri Botanical Garden Annual Report 1906; 1906: 147-152.

Innan H, Terauchi R, Miyashita NT: Microsatellite polymorphism in natural populations of wild plant *Arabidopsis thaliana*. Genetics. 1997; 146: 1441-1452.

Jackson SD: Plant responses to photoperiod. New Phytology. 2009; 181 (3): 517-531. (doi:10.1111/j.1469-8137.2008.02681.x)

Jarne P and Lagoda PJL: Microsatellites, from molecules to populations and back. Trends in Ecol and Evol. 1996; 11: 424-429. (doi:10.1016/0169-5347(96)10049-5)

Jørgensen S and Mauricio R: Neutral genetic variation among wild North American populations of the weedy plant Arabidopsis thaliana is not geographically structured. Molecular Ecology. (2004) 13: 3403–3413 (doi:10.1111/j.1365-294X.2004.02329.x)

Jump AS and Peñuelas J: Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters. 2005; 8: 1010–1020. (doi:10.1111/j.1461-0248.2005.00796.x)

Kaplan I, Dively GP and Denno RF: The costs of anti-herbivore defense traits in agricultural crop plants: a case study involving leafhoppers and trichomes. Ecological Applications. 2009; 19(4): 864–872.

Kawecki TJ and Ebert D: Conceptual issues in local adaptation. Ecology Letter. 2004; 7: 1225–241. (doi: 10.1111/j.1461-0248.2004.00684.x)

Karakaya HC, Tang Y, Cregan PB and Knap HT: Molecular mapping of the fasciation mutation in soybean, *GLYCINE MAX* (LEGUMINOSAE). American Journal of Botany. 2002; 89: 559-565.

Kearsey MJ, Pooni HS and Syed NH: Genetics of quantitative traits in *Arabidopsis thaliana*. Heredity. 2003; 91: 456-464. (doi:10.1038/sj.hdy.6800306)

Kebrom TH and Brutnell TP: The molecular analysis of the shade avoidance syndrome in the grasses has begun. J. Exp. Bot. 2007; 58 (12): 3079-3089. (doi:10.1093/jxb/erm205)

Kim Y and Nielsen R: Linkage disequilibrium as a signature of elective sweeps. Genetics. 2004; 167: 1513-1524 (doi:10.1534/genetics.103.025387)

King RW, Hisamatsu T, Goldschmidt EE and Blundell C: The nature of floral signals in *Arabidopsis*. I. Photosynthesis and a far-red photoresponse independently regulate flowering by increasing expression of *FLOWERING LOCUS T* (*FT*). J Exp Bot. 2008; 59 (14): 3811-3820. (doi:10.1093/jxb/ern231)

Kliebenstein, DJ, Kroymann J, Brown P, Figuth A, Pedersen D, Gershenzon J and Mitchell-Olds T: Genetic control of natural variation in Arabidopsis glucosinolate accumulation. Plant Physiol. 2001; 126, 811–825.

Knox MR and Ellis THN: Stability and inheritance of methylation states at *Pst*I sites in *Pisum*. Mol Genet Genomics. 2001; 265: 497-507. (doi:10.1007/s004380000438)

Knight TM and Miller TE: Local adaptation within a population *Hydrocotyle bonariensis*. Evolutionary Ecol Res. 2004; 6: 103–114

Koopman WJM: Phylogenetic Signal in AFLP Data Sets. *Syst Biol* (2005) 54 (2): 197-217. (doi: 10.1080/10635150590924181)

Koornneef M, Alonso-Blanco C, Peeters AJM and Soppe W: Genetic control of flowering time in Arabidopsis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1998, 49: 345-370(doi:10.1146/annurev.arplant.49.1.345)

Koornneef M, Alonso-Blanco C and Vreugdenhil D: Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu. Rev. Plant Biol. 2004. 55: 141-72. (doi:10.1146/annurev.arplant.55.031903.141605)

Korpf M, Comes HP and Kadereit JW: An AFLP clock for the absolute dating of shallow-time evolutionary history based on the intraspecific divergence of southwestern European alpine plant species. Molecular Ecology. 2009; 18: 697–708. (doi:10.1111/j.1365-294X.2008.04053.x)

Korves TM, Schmid KJ, Caicedo AL, Mays C, Stinchcombe JR, Purugganan MD and Schmitt J: Fitness effects associated with the major flowering time gene FRIGIDA in *Arabidopsis thaliana* in the field. The American Naturalist. 2007; 169 (5): E141-E157. (doi: 10.1086/513111)

Kroymann J and Mitchell-Olds T: Epistasis and balanced polymorphism influencing complex trait variation. Nature. 2005; 435:95–98. (doi:10.1038/nature03480)

Kuittinen H, Mattila A and Savolainen O: Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. Heredity. 1997; 79: 144-152.

Külheim C, Årgen J and Jansson S: Rapid regulation of light harvesting and plant fitness in the field. Science. 2002; 297 (5578): 91-93. (doi:10.1126/science.1072359)

Lee JA: A study of plant competition in relation to development. Evolution. 1960; 14 (1): 18-28.

Leimu R and Fischer M: A meta-analysis of local adaptation in plants. *PLoS One*. 2008; 3 (12): e4010. doi:10.1371/journal.pone.0004010

Leinonen PH, Sandring S, Quilot B, Clauss MJ, Mitchell-Olds T, Ågren J and Savolainen O: Local adaptation in European populations of *Arabidopsis lyrata* (Brassicaceae). American Journal of Botany. 2009; 96 (6): 1129–1137. (doi: 10.3732/ajb.0800080)

Leinonen PH, Remington DL, Leppälä J and Savolainen O: Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. Molecular Ecology. 2012. (doi: 10.1111/j.1365-294X.2012.05678.x)

Li Y-C, Röder MS, Fahima T, Kirzhner VM, Beiles A, Korol AB and Nevo E: Climatic effects on microsatellite diversity in wild emmer wheat (Triticum dicoccoides) at the Yehudiyya microsite, Israel. Heredity. 2002; 89: 127–132. (doi:10.1038/sj.hdy.6800115)

Linhart YB and Grant MC: Evolutionary significance of local genetic differentiation in plants. Annu. Rev. Ecol. Syst. 1996; 27: 237-277. (doi:10.1146/annurev.ecolsys.27.1.237)

Lippman ZB and Zamir D: Heterosis: revisiting the magic. *Trends Genet*. 2007; 23 (2): 60-66. (doi:10.1016/j.tig.2006.12.006)

Lütz C: Cell physiology of plants growing in cold environments. Protoplasma. 2010; (244): 53–73. (doi:10.1007/s00709-010-0161-5)

Mackay TF: The genetic architecture of quantitative traits. Annu Rev Genet. 2001; 35: 303-339. (doi: 10.1146/annurev.genet.35.102401.090633)

Maina GG, Brown JS and Gersani M: Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. Plant Ecology. 2002; 160: 235-247.

Marschall E, Jogler, M, Henßge, U and Overmann J: Large-scale distribution and activity patterns of an extremely low-light-adapted population of green sulfur bacteria in the Black Sea. Environmental Microbiology. 2010; 12: 1348–1362. (doi:10.1111/j.1462-2920.2010.02178.x)

The Mathworks Incorporated; MATLAB v7.0.4.365 SP2; http://www.mathworks.com/

McMillan C: Photoperiodic adaptation in desert populations of Xanthium strumarium. Am J Bot. 1973; 60(5): 397-405.

McWilliam JR and Naylor AW: Temperature and plant adaptation. I. Interaction of temperature and light in the synthesis of chlorophyll in corn. Plant Physiol. 1967; (42): 1711-1715.

Miyashita NT, Kawabe A and Innan H: DNA variation in the wild plant *Arabidopsis thaliana* revealed by amplified fragment length polymorphism analysis. Genetics. 1999; 152: 1723–1731.

Malo JE, Jiménez B and Suárez F: Seed bank build-up in small disturbances in a Mediterranean pasture: the contribution of endozoochorous dispersal by rabbits. Ecography. 1995; 18 (1): 73-82. (doi:10.1111/j.1600-0587.1995.tb00120.x)

Maloof JN, Borevitz JO, Dabi T, Lutes J, Nehring RB, Redfern JL, Trainer GT, Wilson JM, Asami T, Berry CC, Weigel D and Chory J: Natural variation in light sensitivity of Arabidopsis. Nature Genetics. 2001; 29:441-446. (doi:10.1038/ng777)

Maloof JN: QTL for Plant growth and morphology. Current Opinion in Plant Biology. 2003; 6: 85-90. (doi:10.1016/S1369526602000080)

Marshall DL, Fowler NL and Levin DA: Plasticity in Yield Components in Natural Populations of Three Species of Sesbania. Ecology. 1985; 66 (3): 753-761.

Masclaux-Daubresse C, Purdy S, Lemaitre T, Pourthau N, Taconnat L, Renou JP and Wingler A: Genetic Variation Suggests Interaction between Cold Acclimation and Metabolic Regulation of Leaf Senescence. Plant Physiology. 2007; 143: 434-446. (doi:10.1104/pp.106.091355)

Matsumoto-Kitanoa M, Kucumotoa T, Tarkowskib P, Kinoshita-Tsujimuraa K, Václavíkováb K, Miyawakia K and Kakimoto T: Cytokinins are central regulators of cambial activity. PNAS. 2008; 105 (50): 20027-20031. (doi:10.1073/pnas.0805619105)

McGraw JB and Garbutt K: The analysis of plant growth in ecological and evolutionary studies. Tree. 1990; 5 (8): 251-254.

Méndez-Vigo B, de Andrés MT, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C: Temporal analysis of natural variation for the rate of leaf production and its relationship with flowering initiation in *Arabidopsis thaliana*. J Exp Bot. 2010; 61(6): 1611-23. (doi:10.1093/jxb/erq032)

Mertens TR and Burdick AB: The Morphology, Anatomy, and Genetics of a Stem Fasciation in *Lycopersicon esculentum*. Am J Bot. 1954; 41 (9): 726-732.

Met Office 1, United Kingdom, accessed June 2012 (http://www.metoffice.gov.uk/climate/uk/es/)

Met Office 2, United Kingdom, accessed June 2012 http://www.metoffice.gov.uk/education/teens/case-studies/indiansummer

Miles CM and Wayne M: Quantitative trait locus (QTL) analysis. Nature Education. 2008; 1 (1).

Munguía-Rosas MA, Ollerton J, Parra-Tabla V and De-Nova JA: Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. Ecology Letters. 2011; 14: 511-521. (doi: 10.1111/j.1461-0248.2011.01601.x)

Nei M: Genetic distance between populations. The American Naturalist. 1972; 106 (949): 283-292.

Noodén LD, Hillsberg JW and Schneider MJ: Induction of leaf senescence in *Arabidopsis thaliana* by long days through a light-dosage effect. Physiologia Plantarum. 1996; 96 (3): 491-495. (doi: 10.1111/j.1399-3054.1996.tb00463.x)

Nordborg M and Tavare S: Linkage disequilibrium: what history has to tell us. TRENDS in Genetics. 2002; 18:83-90.

Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA and Weigel D: The extent of linkage disequilibrium in *Arabidopsis thaliana*. Nature genetics. 2002; 30:190-193.

Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R, Jakobsson M, Kim S, Morozov Y, Padhukasahasram B, Plagnol V, Rosenberg NA, Shah C, Wall JD, Wang J, Zhao K, Kalbfleisch T, Schulz V, Kreitman M and Bergelson J: The Pattern of Polymorphism in *Arabidopsis thaliana*. PLoS Biology. 2005; 3(7):1289-1299.
Novoplansky A: Picking battles wisely: plant behaviour under competition. Plant, Cell and Environment. 2009; 32: 726-741. (doi: 10.1111/j.1365-3040.2009.01979.x)

O'Brien EE, Gersani M, Brown JS: Root proliferation and seed yield in response to spatial heterogeneity of below-ground competition. New Phytologist. 2005; 168 (2): 401-412. (doi: 10.1111/j.1469-8137.2005.01520.x)

Olatunde Akinola M, Thompson K and Buckland S: Soil seed bank of an upland calcerous grassland after 6 years of climate and management manipulations. J Appl Ecol. 1998; 35: 544–552. (doi: 10.1046/j.1365-2664.1998.3540544.x)

Ortiz R, Freyre R, Peloquin SJ and Iwanaga M: Adaptation to day length and yield stability of families from 4x × 2x crosses in potato. Euphytica. 1999; 56: 187-195. (doi:10.1007/BF00042062)

Peng J, Korol AB, Fahima T, Röder MS, Ronin YI, Li YC and Nevo E: Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: Genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res. 2000; 10:1509-1531. (doi:10.1101/gr.150300)

Picó FX, Méndez-Vigo B, Martínez-Zapater JM and Alonso-Blanco C: Natural genetic variation of Arabidopsis thaliana is geographically structure in the Iberian Peninsula. Genetics. 2008; 180: 1009–1021. (doi:10.1534/genetics.108.089581)

Pieters GA, Van Den Noort ME and Van Nijkerken JA: Growth adaptation of leaves and internodes of poplar to irradiance, day length and temperature. Tree Physiology. 1999; (19) 933-942.

Pigliucci M, Whitton J and Schlichting CD: Reaction norms of Arabidopsis. I. Plasticity of characters and correlations across water, nutrient and light gradients. J Evol Biol. 1995; 8:421 – 438.

Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, Ågren J, Bossdorf O, Byers D, Donohue K, Dunning M, Holub EB, Hudson A, Le Corre V, Loudet O, Roux F, Warthmann N, Weigel D, Rivero L, Scholl R, Nordborg M, Bergelson J and Borevitz JO: The scale of population structure in *Arabidopsis thaliana*. PLoS Genetics. 2010; 6 (2): e1000843. (doi:10.1371/journal.pgen.1000843)

Pouteau S and Albertin C: The significance of bolting and floral transitions as indicators of reproductive phase change in Arabidopsis. J Exp Bot. 2009; 60 (12): 3367-3377. (doi:10.1093/jxb/erp173)

Powell W, Thomas WTB, Baird E, Lawrence P, Booth A, Harrower B, McNicol JW and Waug R: Analysis of quantitative traits in barley by the use of Amplified Fragment Length Polymorphisms. Heredity. 1997; 79: 48-59. (doi:10.1038/hdy.1997.122)

Roach DA and Wulff RD: Maternal effects in plants. Annu Rev Ecol Syst. 1987; 18: 209-235.

Roberts HA: The changing population of viable weed seeds in an arable soil. Weed Research. 1968; 8 (3): 253–256. (doi: 10.1111/j.1365-3180.1968.tb01428.x)

Rutter MT and Fenster CB: Testing for Adaptation in *Arabidopsis thaliana*: A Calibrated Common Garden Approach. Annals of Botany. 2007; 99: 529–536. (doi:10.1093/aob/mcl282)

Sambatti JBM and Rice KJ: Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). Evolution. 2006; 60 (4): 696-710. (doi:http://dx.doi.org/10.1554/05-479.1)

Schemske DW: Population structure and local selection in *Impatiens pallida* (Balsaminaceae), A selfing annual. Evolution. 1984; 38 (4): 817–832.

Sharbel TF, Haubold B and Mitchell-Olds T: Genetic isolation by distance in Arabidopsis thaliana. Molecular Ecology. 2000; 9: 2109–2118.

Shimizu KK and Purugganan MD: Evolutionary and Ecological Genomics of Arabidopsis. Plant Physiology. 2005; 138:578-584

Shindo C, Bernasconi G and Hardtke C: Natural Genetic Variation in Arabidopsis: Tools, Traits and Prospects for Evolutionary Ecology. Annals of Botany. 2007; 99:1043-1054.

Silvertown J: Plants coexistence and the niches. Trends Ecol. Evol. 2004; 19 (11): 605-611. (doi:10.1016/j.tree.2004.09.003)

Simpson GG and Dean C: Arabidopsis, the rosette stone of flowering time. Science. 2002; 296: 285-289. (doi:10.1126/science.296.5566.285)

Slatkin M: Isolation by distance in equilibrium and non-equilibrium populations. Evolution. 1993; 47 (1): 264-279.

Smallwood M and Bowles DJ: Plants in a cold climate. Phil Trans. R. Soc. Lond. 2002; 357: 831-847

Smith H and Whitelam GC: The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant, Cell & Environment. 1997; 20: 840-844. (doi:10.1046/j.1365-3040.1997.d01-104.x)

Sokal RR and Rohlf FJ: Introduction to Biostatistics. W.H. Freeman and Company, New York 2nd edition. 1987; 133-270.

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller HL (eds.): Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 2007.

Sork VL, Stowe KA, Hochwender C: Evidence for local adaptation in closely adjacent subpopulations of Northern Red Oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. The American Naturalist. 1993; 142 (6): 928-936.

Stenøien HK, Fenster CB, Tonteri A and Savolainen O: Genetic variability in natural populations of Arabidopsis thaliana in northern Europe. Molecular Ecology. 2005; 14: 137-148. (doi: 10.1111/j.1365-294X.2004.02359.x)

Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD and Schmitt J: A latitudinal cline in flowering time in Arabidopsis thaliana modulated by the flowering time gene FRIGIDA. PNAS. 2004; 101 (13) 4712-2717. (doi:10.1073/pnas.0306401101)

Tanksley SD: Mapping polygenes. Annual Review of Genetics. 1993; 27: 205-233. (doi:10.1146/annurev.ge.27.120193.001225)

Thimann KV and Sachs T: The Role of Cytokinins in the "Fasciation" Disease Caused by *Corynebacterium fascians*. Am J Bot. 1966; 53 (7): 731-739.

Todokoro S, Terauchi R and Kawano S: Microsatellite polymorphisms in natural populations of *Arabidopsis thaliana* in Japan. Jpn. J. Genet. 1996; 70: 543–554.

Toomajian C, Hu TT, Aranzana MJ, Lister C, Tang C, Zheng H, Zhao K, Calabrese P, Dean C and Nordborg M: A nonparametric test reveals selection for rapid flowering in th Arabidopsis genome. PLoS Biol. 2006; 4 (5): e137. (doi: 10.1371/journal.pbio.0040137)

Tian D, Traw MB, Chen JQ, Kreitman M and Bergelson J: Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. Nature. 2003; 423: 74-77. (doi:10.1038/nature01588)

Tremmel CD and Bazzaz FA: Plant architecture and allocation in different neighborhoods: Implications for competitive success. Ecology. 1995; 76 (1): 262-271.

Tsukaya H: Mechanism of leaf-shape determination. Annu Rev Plant Biol. 2006; 57: 477-496. (doi:10.1146/annurev.arplant.57.032905.105320)

Turesson G: The plant species in relation to habitat and climate. Hereditas. 1925; 6 (2): 147-236. (doi: 10.1111/j.1601-5223.1925.tb03139.x)

Turesson G: The genotypical response of the plant species to the habitat. Hereditas. 1922; 3 (3): 211-350 (doi: 10.1111/j.1601-5223.1922.tb02734.x)

Tyrka 2002: A simplified AFLP method for fingerprinting of common wheat (*Triticum aestivum* L.) cultivars

Ungerera MC, Halldorsdottira SS, Modliszewskia JL, Mackay TFC, and Purugganan MD: Quantitative Trait Loci for Inflorescence Development in *Arabidopsis thaliana*. Genetics. 2002; 160: 1133-1151

Vekemans X; AFLP-*SURV* 1.0 http://www.ulb.ac.be/sciences/lagev/aflp-surv.html

Verhoeven KJF, Poorter H, Nevo E and Biere A: Habitat-specific natural selection at a flowering-time QTL is a main driver of local adaptation in two wild barley populations. Molecular Ecology. 2008; 17: 3416-3424 (doi: 10.1111/j.1365-294X.2008.03847.x)

Volis S: Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. Evol Ecol. 2007; 21: 381–393. (doi:10.1007/s10682-006-9108-x)

Volis S, Mendlinger S and Ward D: Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history

and local adaptation. Biological Journal of the Linnean Society. 2002; 77 (4): 479-490. (doi: 10.1046/j.1095-8312.2002.00120.x)

Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M: AFLP: a new technique for DNA fingerprinting. Acids Res. 1995; 23: 4407–4414.

Weigel D and Nordborg M: Natural variation in Arabidopsis- how do we find the causal genes. Plant Physiology. 2005; 138: 567-568

Weiner J and Thomas SC: Competition and allometry in three species of annual plants. Ecology. 1992; 73 (2): 648-656. (doi.10.2307/1940771)

Weinig C, Ungerer MC, Dorn LA, Kane NC, Toyonaga Y, Mackay TFC, Purugganan MD, Schmitt J: Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. Genetics. 2002; 162:1875–84.
Wentworth M, Murchie EH, Gray JE, Villegas D, Pastenes C, Pinto M, and Horton P: Differential adaptation of two varieties of common bean to abiotic stress - II. Acclimation of photosynthesis. JXB. 2006; 57 (3): 699–709. (doi:10.1093/jxb/erj061)

Weising K, Nybom Hilde, Pfenninger M, Wolff K and Kahl G: DNA fingerprinting in plants: Principles, methods and applications. Taylor and Francis Group 2nd edition. 2005.

Wilcox CD, Dove SB, McDavid WB and GreerDB; UTHSCSA ImageTool v3.0. http://ddsdx.uthscsa.edu/dig/itdesc.html

Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker A, Anderson J, Egan JF, Moyers BT, Petipas R, Giakountis A, Charbit E, Coupland G, Welch SM and Schmitt J: Effects of genetic perturbation on seasonal life history plasticity. Science. 2009; 323 (5916): 930-934 (doi:10.1126/science.1165826)

Willis CG, Brock MT and Weinig C: Genetic variation in tolerance of competition and neighbour suppression in *Arabidopsis thaliana*. J. Evol. Biol. 2010; 23: 1412-1424. (doi:10.1111/j.1420-9101.2010.02003.x)

Wilson AJ, Coltman DW, Pemberton JM, Overall ADJ, Bryne KA and Kruuk LEB: Maternal genetic effects set the potential for evolution in a free-living vertebrate population. J Evol Biol. 2005; 18: 405-414. (doi:10.1111/j.1420-9101.2004.00824.x)

Wilson JB: Shoot competition and root competition. J. Appl Ecol. 1988; 25 (1): 279-296.

Winn AA and Werner PA: Regulation of seed yield within and among populations of *Prunella Vulgaris*. Ecology. 1987; 68 (5): 1224-1233.

Wittstock U and Halkier BA: Glucosinolate research in the Arabidopsis era. TRENDS in Plant Science. 2002; 7 (6): 263-270.

White OF: Fasciation. Botanical Review. 1948; 14 (6): 319-358.

Wright S: Isolation by distance. Genetics. 1943; 28: 114–138.

Wu HP: Genetic basis of plant stability in *Arabidopsis thaliana*. Bot. Bull. Academia Sinica. 1972; 13: 29-36.

Zeevaart JA: Leaf-produced floral signals. Curr Opin Plant Biol. 2008; 11(5): 541-547. (doi: 10.1016/j.pbi.2008.06.009)